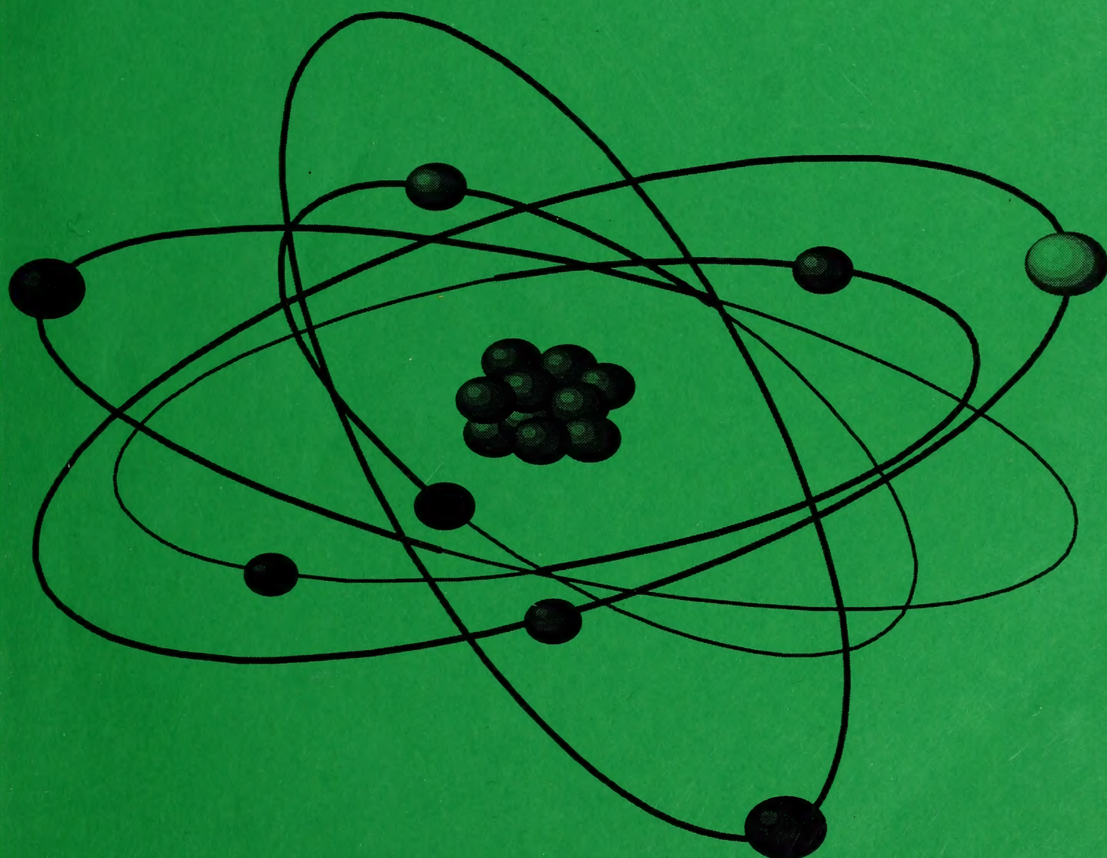
30TH ANNUAL WESTERN TRACE ORGANIC AND PESTICIDE RESIDUE WORKSHOP

EDMONTON, ALBERTA 1995



30TH ANNUAL WESTERN TRACE ORGANIC AND PESTICIDE RESIDUE WORKSHOP

EDMONTON, ALBERTA, MAY 8 & 9, 1995

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CHLOROFLUOROCARBONS (CFCs) IN AEROSOL PRODUCTS

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Environmental Protection Branch
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ABSTRACT

In 1987, Canada and 24 other nations signed the Montreal Protocol which committed them to control substances that deplete the ozone layer. Subsequently, in 1990 and 1993 Canada developed regulations under the Canadian Environmental Protection Act aimed at controlling ozone depleting substances. In order to enforce these Regulations, methods were needed to determine the presence of CFCs in pressurized aerosol containers. Environment Canada's Edmonton laboratory has developed a method for sampling pressurized aerosol containers and for measuring the CFC content using gas chromatography - mass spectrometry (GC/MS).

INTRODUCTION

Traditionally, aerosol products such as hair spray, refrigerants, deodorants, electronic cleaning agents have used Chlorofluorocarbons (CFCs) as propellants and solvents to dissolve and dispense the active ingredients from the aerosol cans. With the enactment of the Ozone-depleting Substances Products Regulations under the Canadian Environmental Protection Act (CEPA) in 1990, the availability of CFCs for such purposes was controlled. The enforcement of these Regulations required the development of a method to determine the presence of the restricted CFCs in this type of product. The following method was developed to sample and test for Trichlorofluoromethane (CFC-11), Dichlorodifluoromethane (CFC-12), 1,1,2-Trichlorotrifluoroethane (CFC-113) and 1,2-Dichlorotetrafluoroethane (CFC-114) which were most commonly used in aerosol containers.

METHOD

An Aerosol Can Sampling Apparatus (Figure 1) was used to obtain a representative gaseous phase sample for analysis. The apparatus consists of tygon and glass tubing, a solvent trap, a gas sampling bulb and a methanol reservoir to seal the system from air.

In cases where the standards or samples required dilution, an aliquot was transferred from the original gas sampling bulb to a second bulb containing nitrogen. Nitrogen in this second bulb acts as the diluent.

The sampling apparatus was initially flushed with nitrogen gas to remove any contaminants. The aerosol containers were inverted and the gaseous phase contents dispensed into the sampling apparatus. The sample contents were dispensed for a time period sufficient to completely flush the sampling bulb and thus obtain a representative sample of the container contents. The CFC standards, which are contained in lecture bottles, were sub-sampled in the same manner.

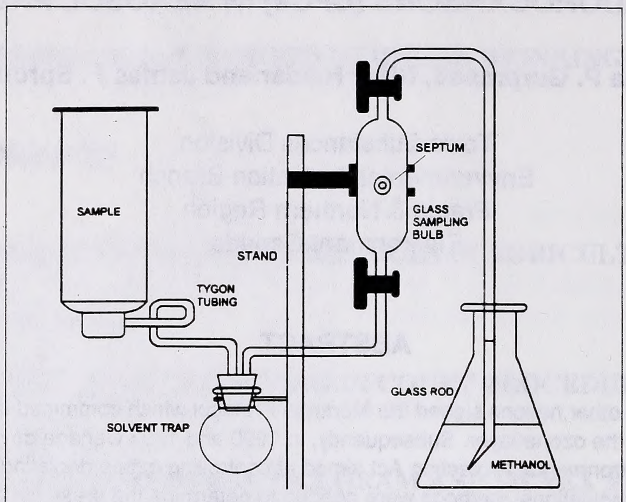


FIGURE 1

Due to the risk of cross contamination, dedicated syringes and gas sampling bulbs were used for each individual sample and standard.

For quality control purposes, blanks and replicates were prepared for analysis in the same manner. A blank was analysed with each sample set, to check for cross contamination.

INSTRUMENTAL ANALYSIS

A gas chromatographic/mass spectrometric (GC/MS) method was used for the analysis of (CFC-11), (CFC-12) (CFC-113) and (CFC-114) in the samples.

Analysis was carried out using a Hewlett-Packard (HP5971) GC/MS system in the electron impact mode, scanning 50 to 180 amu. For the GC, a 105 m, Rtx 502.2, 2 micron film thickness, 0.32 mm I.D., column was utilized with helium carrier gas at 28 psi head pressure. The split injector (50:1) was set at 250° C with the GC temperature programmed at 50° C isothermal for 15 min.. The transfer line was set at 280° C.

A measured amount of sample was injected into the GC/MS system. For the standards and samples analysed, the characteristic ions (m/z 101,103 for CFC-11, m/z 85,87 for CFC-12, m/z 151,153 for CFC-113 and m/z 135,137 for CFC-114) were integrated for quantitation.

RESULTS AND DISCUSSION

The analysis of CFC-12, CFC-11, CFC-113 and CFC-114 in the aerosol products was easily carried out. The chromatographic peaks were well separated and identification of the CFCs was easily done by comparing mass spectras (Figure 2).

In terms of quantitative analysis, reanalysis of the same sample at a later time (days or months later) showed significant changes in concentration (Table 1). Much smaller variations in CFC concentration were found when replicate analyses of the same container were carried out on the same day. Different cans of the same product lot also showed little variation in

concentration (Table 2). These changes in concentration over time can be attributed to the difference in equilibrium constants of the various components in the containers. As the aerosol can loses pressure because of leakage and/or the withdrawal of samples, the concentrations of the various components in both the liquid and gas phase change.

Table 1:
An aerosol can containing F113 analysed 4 different times during a 2 month period

Concentrations (μg/cc)	764, 957, 1347, 149
%RSD	62

Table 2:
Precision of 6 separate aerosol cans of the same product lot containing F113

Concentrations (μg/cc)	1183, 1019, 987, 1134, 1437, 1719
%RSD	23

The precision of the standards used was quite good at all times (Table 3). Out of 281 aerosol products tested, 87 were found positive for CFC (Table 4).

Table 3:
Precision of the CFCs over 30 days & repeated 5 times

Chloroflorocarbons	%RSD
F12	5.3
F114	17
F11	9.0
F113	12

Table 4:
Examples of the type of products, number of positives and CFCs found

Product	Samples	Positives	CFCs found
Hair spray	48	1	F11
Lubricants	25	15	F11, F113, F12
Lock de Icer	8	0	Nil
Electronic Cleaners	17	10	F113

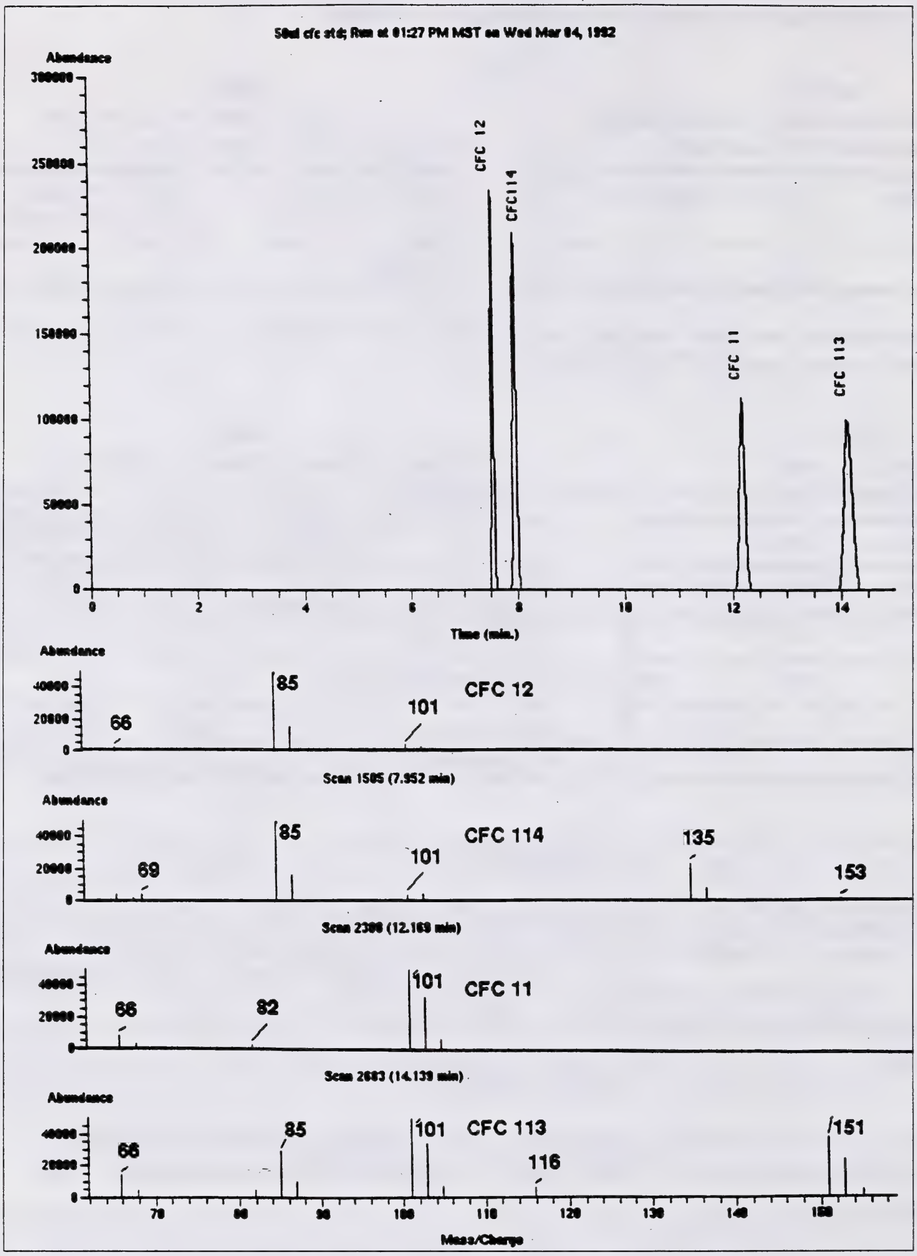
CONCLUSION

Although the quantitation of CFCs in some aerosol products has some drawbacks in using this method, it is adequate for enforcement of the Ozone-depleting Substances Products Regulations. The regulations are designed to eliminate the use of CFCs in aerosol products and as such the regulations are broached if any quantity of CFC is present.

REFERENCES

R.A.F. Matheson and J.J. Wrench, Analysis of Chlorofluorocarbons in Aerosol Products, Environmental Protection Service, Atlantic Region, Dartmouth, Nova Scotia, August, 1982, EPS-4-AR-83-1.

Figure 2: Total Ion Chromatogram and Mass Spectras of CFC11, CFC12, CFC113 and CFC114



MULTI-RESIDUE METHOD FOR THE DETERMINATION OF PESTICIDES IN FRUITS AND VEGETABLES USING GC/MSD AND HPLC/FLUORESCENCE DETECTION

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OVERVIEW

The Agriculture & Agri-Food Canada Lab in Calgary receives a variety of fruit and vegetable samples for pesticide residue analysis to ensure compliance with the Food & Drug Act. Our lab screens each sample for 200 pesticides, to determine whether or not any pesticides present in that sample exceed the allowable Maximum Residue Limit, as established by Health Canada.

Following is a single extraction method that will analyze 190 pesticides by GC/MSD, and 10 carbamates by HPLC with post-column derivitization and Fluorescence detection.

METHOD

EXTRACTION

1) 50.0 grams of homogenized sample is weighed into a 500 mL mason jar.
2) Add 100 mL of acetonitrile, and blend on an Omni-Mixer for 2 minutes.
3) Add 20 grams of NaCl, and Omni-mix for another 2 minutes.
4) Transfer the entire blended sample to a 250 mL centrifuge bottle, stopper and centrifuge at 1000 rpm for 4 minutes.
5) Pour most of the top organic layer into an erlenmeyer flask containing ~ 15 grams of dried powdered sodium sulfate.
6) Swirl the erlenmeyer well a few times, then allow it to sit and clarify for a few minutes.
7) Transfer 30.0 mL of the liquid extract into a Turbovap tube and concentrate to approx. 0.5 mL. The sample is now ready for clean-up.

CLEAN-UP

For the clean-up, Envi-Carb cartridges from Supelco are attached in series with Aminopropyl Sep Paks from Waters. The samples are allowed to pass through the two in tandem by gravity.

1) ~3 cm of dried granular sodium sulfate is added to the Envi-Carb cartridge, and an Aminopropyl sep pak is attached to the tip of the cartridge. These are set into a holding ring on top of a Turbovap tube.
2) The cleanup tubes are pre-rinsed with 3-4 mL of 3:1 acetonitrile:toluene.

3) The concentrated sample extract is transferred to the cleanup columns, and the pesticides are eluted with 25 mL of 3:1 acetonitrile:toluene.
4) The eluant is concentrated under Nitrogen and solvent exchanged to acetone.
5) Each sample extract is split for carbamate and multi-residue analyis.
6) The carbamate portion is solvent exchanged to methanol under Nitrogen, and brought down to 0.5 mL. The final volume is then adjusted with pH=3 Milli-Q water, and the sample is analyzed by HPLC with post-column derivitization and Fluorescence detection.

The multi-residue portion is brought to its final volume with acetone, internal standard is added, and the sample is run on 2 GC/MSDs, each screening for half of the 190 pesticides, using 'Selected Ion Monitoring' methods. Data Analysis is performed using Target software.

CONCLUSION

This method has been used for the majority of this past year's samples, with much success. 85-90% of the compounds are recovered in amounts of >70%. The extracts are sufficiently clean that our instrument maintenance has been reduced significantly from previously used methods. While sample matrix type seems to affect the recovery of certain pesticides significantly, it has been found that this clean-up system works well for minimizing interfering sample co-extractives in a large variety of fruits and vegetables. Our lab has so far evaluated this method on the following commodities: strawberries, grapes, beans, celery, corn, kiwi, peas, pineapples, potatoes, pumpkins and tomatoes, and in the coming year, will be tested on a total of 43 different fruits and vegetables.

Our lab generally runs batches of 20-25 samples per day, plus spikes and blanks.

MULTIRESIDUE ANALYSIS: A MODULAR APPROACH TO HANDLE DIFFICULT MATRICES

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Our current multiresidue method for the analysis of fruits and vegetables consists of an acetonitrile extraction, followed by a salting out step and a charcoal:celite clean-up. The clean-up has recently been modified and we now use an Envicarb™/aminopropyl clean-up. The analysis is performed by GC/MSD in selected ion monitoring (SIM) mode, and by HPLC with post column fluorescence detection for the carbamates. This protocol allows us to analyze for 200 pesticides.

Our method can be adapted very easily to handle difficult commodities. In some cases, the acetonitrile extraction step is replaced by an acetone extraction followed by a partition. The clean-up is the same. Oily matrices, such as corn, require a gel permeation chromatography clean-up. Citrus fruits present a particular problem for the analysis of carbamates and also require an additional clean-up.

The method can also be optimized to handle specific analytes. Over 90% of the compounds elute from the clean-up column in the first 5 mL of solvent, but the regular method calls for a 20 mL elution volume for good recovery of the 13 other pesticides. It is possible to take advantage of this for the analysis of surveillance or compliance samples, where speed of analysis is critical and the analysis requested is typically for one or two compounds only. The elution volume can be lowered to elute the analyte(s) of interest, thereby considerably speeding up the method and yielding even cleaner extracts.

Over years of multiresidue analysis, our laboratory has gained experience with many commodities. The vast majority of them can be handled by the regular procedure. But for the few matrices that require special extraction or clean-up steps, it can be useful to look at the method in a modular way. Some of the factors to consider are the type of commodity to analyze, previous experience with similar matrices and the number of samples expected. The method can be optimized for each commodity by determining which extraction procedure is best suited and which clean-up(s) is/are required. In all cases, the analysis is performed the same way and covers all 200 compounds. Recovery data is also generated for every set. This approach allows for the optimization of resources available in the laboratory.

Multiresidue analysis: a modular approach to handle difficult matrices

Julie Fillion

Agriculture and Agrifood Canada

Pesticides Monitoring & Surveillance Unit

- Monitoring samples
  - large number of samples, 200 pesticides
  - expertise in handling different matrices
  - specific analysis also required
- Surveillance samples
  - fewer samples
  - 1-2 analytes
  - fast turnaround time required

Characteristics of the ideal multiresidue method

- Covers a wide range of pesticides
- Rapid and sensitive
- Good recoveries and accurate quantitation
- Applicable to different types of commodities
- Easily adapted to handle difficult matrices
- Easily adapted to handle specific analytes

Two different aspects to consider

- Adapting the method to handle difficult matrices
- Adapting the method to specific analytes, in order to speed up the process

Current method

50 g sample + 100 mL ACN
10 g NaCl

↓

10 mL aliquot
Envicarb/Aminopropyl clean-up
20 mL ACN:toluene 3:1

↓

MSD analysis (189 pesticides) | HPLC analysis (10 carbamates)

Some problem commodities

- Citrus fruits
  - naturally fluorescent compounds detected on HPLC
  - late eluters on GC
- Corn
  - oily matrix
- Apples
  - wax causes problems
- Cranberries
  - particularly dirty

Modular approach to matrix problems

- Extraction: acetonitrile
  acetone
- Clean-up(s): partition
  gel permeation chromatography
  solid phase extraction
- Analysis

Acetonitrile vs acetone as extraction solvent

- ACN extraction
  - simple salting out step to get rid of water
  - aqueous ACN has a very high solvating power
  - more coextractives than with acetone
- Acetone extraction
  - partition is required
  - high solvent use
  - small sample throughput
  - less coextractives

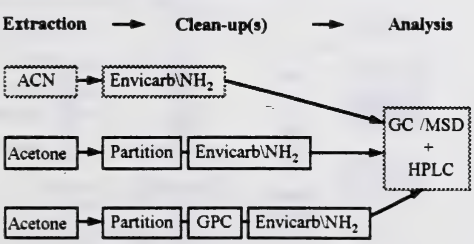
Comparison of different clean-ups

- Charcoal:celite — loss of certain compounds
- Envicarb only — extracts not sufficiently clean
- Envicarb/NH_2 — good clean-up + recoveries; some problem commodities (?)
- C_{18} — good clean-up + recoveries
- Partition — high solvent consumption; chlorinated solvents are used; low sample throughput
- GPC — high solvent consumption; chlorinated solvents are used

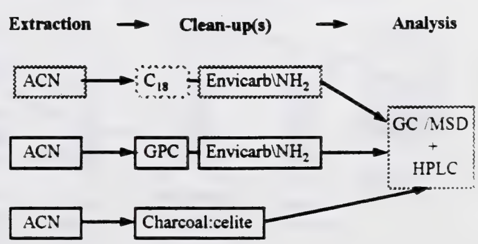
Table of comparison of various clean-ups

Technique	Charcoal celite	Envicarb	Envicarb $+NH_2$	C_{18}	Partition	GPC
Clean-up	good	average	good	good	average	good
Recoveries	some loss	good	good	good	good	good
Solvent use	low	v. low	v. low	v. low	high	high
Cl solvents	none	none	none	none	yes	yes
Sample throughput	high	high	high	high	low	high
Ease of use	good	v. good	v. good	good	poor	average

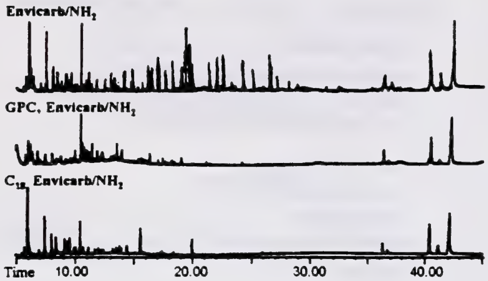
Approaches currently used



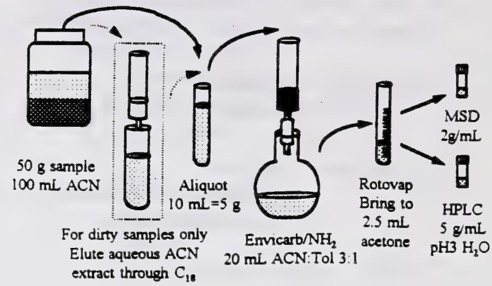
Other options to consider



Comparison of different clean-ups for oranges



Method for clean versus dirty matrices



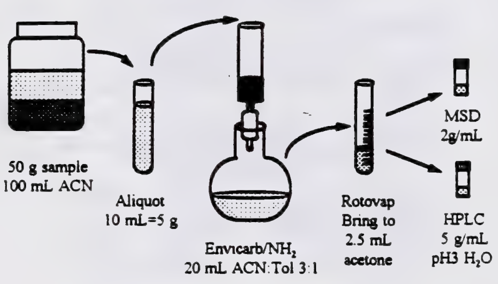
Adapting the method for specific analytes

- Most compounds elute from the SPE columns with 5 mL of solvent
- Size of aliquot can be adjusted in order to speed up analysis
- This can be used to speed up the analysis for surveillance samples

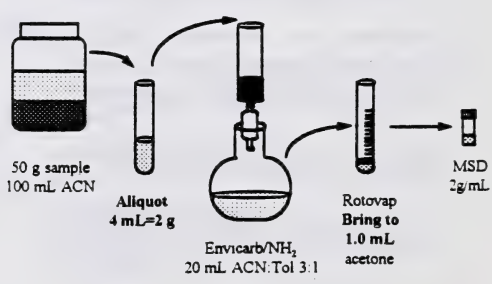
Elution profile Envicarb/NH_2 ACN/Tol 3:1

Elution volume	# compounds recovered
5 mL	187
10 mL	7
15 mL	3
20 mL	1
25 mL	1

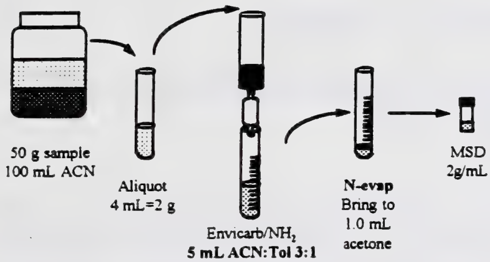
Sample workup for routine samples



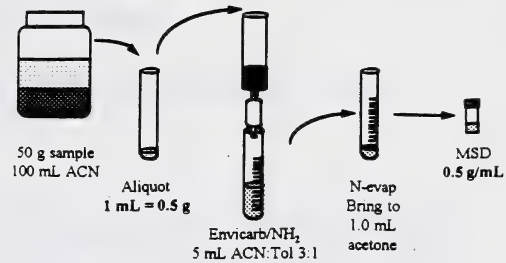
Case #1: Carbamates analysis is not required



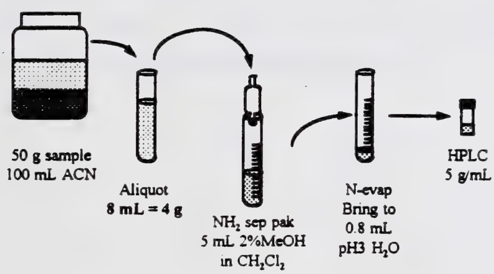
Case #2: analyte elutes with 5 mL of solvent



Case #3: MRL is fairly high (eg. 5 ppm)



Case #4: only carbamate analysis is required



In summary

- Method allows for a versatile approach
- Modular approach is easy to manage
- The best clean-up for each matrix is used
- Always cover 200 compounds (unless analysis of only a few analytes is requested)
- Maximized efficiency for the laboratory

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QUANTITATIVE ANALYSIS OF TOXAPHENE BY GC/MS: A COMPARATIVE STUDY OF DIFFERENT TECHNIQUES

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Although toxaphene occurrence has been found to be widespread in many samples, its analysis still presents a challenge to chemists. electron capture negative ion mass spectrometry (ECNI-MS) has been recognized as the most sensitive and specific method. Recent analysis of several purified individual congeners indicated that the production of [M-Cl] from some congeners with chlorine substitutions at 2,2,5,5-positions were highly susceptible to ECNI conditions thus may result in very weak or totally absent responses in SIM mode. On the other had, electron impact ionization combined with HRMS produces responses (using m/z 159, 161, and 163) similar to that from the GC-ECD for all congeners. Evidence will be present to illustrate that 2,2,5,5-substituted nonachlorobornane which was barely detected by ECNI in fact is one of the abundant congeners in cod, liver, oil, fish, human ad as well as monkey plasma/tissues samples in our feeding studies. Several MS techniques will be compared and difficulties related to trace analysis of toxaphene will be discussed.

COMPARISON OF 2 ANALYTICAL APPROACHES FOR THE ANALYSIS OF ACID HERBICIDES AND METABOLITES

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Traditional clean-up using liquid-liquid partitioning techniques are compared to more recent technology employing solid phase extraction for phenoxy herbicide analysis of soils. Sample throughput, method ruggedness, solvent and glassware usage and subsampling are discussed. Recovery data using both procedures are also compared.

A GRADIENT LC/MS METHOD FOR THE DETECTION OF SULFONAMIDES

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INTRODUCTION

Sulfonamide antibiotics, used by farmers, will often end up as unwanted residues in milk or meat. Agriculture Canada has suggested routine testing for these compounds in a number of imported and domestic consumable products. The accepted testing method has been by HPTLC for some time now, even though little improvement has been made in this time consuming and labour intensive method.

In an attempt to devise a more practical method for the analysis of sulfonamides and also to put to use some of the more advanced technology, we set out to develop an HPLC method using standard apparati which would provide a quick and simple quantitation in a minimal amount of time. This proved to be a relatively easy task and we soon found that we could easily analyze for 4 different sulfa compounds in meat and milk products using an isocratic LC system with UV detection at 265 nm, plus a fifth sulfonamide (Sulfachlorpyridazine) as an internal standard. This procedure provided suitable recoveries of 70 - 80 %, plus detection limits in the low ppb range.

We did, however, want better confirmation of the analytes since chromatograms often gave a number of interferences with UV detection, plus we wanted to screen for a larger number of sulfonamides in a single run. We therefore developed a gradient method using a buffer (Ammonium Acetate) suitable for LC/MS applications. By this method we are able to separate 10 different sulfa compounds in a single 20 minute run. This paper describes the journey we under took to achieve this goal.

MATERIALS AND METHODS

Solvents: All sovents used in the experiments described here were Accusolv Grade available from Anachemia Science (Richmond, B.C.). Water was purified in a Barnstead NANOpure system which showed a resistance of 18.0 MicroOhms/cm.

Reagents: Ammonium Acetate was an HPLC grade purchased from Fisher Scientific (Vancouver, B.C.).

Standards: All sulfonamide standards were purchased from Sigma Chemicals (St. Louis, Mo., U.S.A.) who claimed these products were 99% pure. Note: No additonal peaks were observed in any of the single standard runs on the diodarray detector (DAD) and we therefore assume this claim to be correct.

LC/DAD/MS: The system used in this study was a new Hewlett-Packard instrument purchased in April, 1995. It includes a 1090 (DR type) binary LC pump capable of accurately delivering 1 ul per minute. A 79883A Diodarray Detector, controlled by an HP chemstation running rev A.03.01 HPLC 3D chemstation software. The unit just described is all that is required for the UV data collected in this study.

The MS data was obtained using an API-Electrospray LC/MS system which consists of the instruments just mentioned, coupled to an API-elelctrospray instrument plus a 5898B Mass Spectrometer, more commonly known as the HP MS Engine. This was controlled by an HP chemstation running G1034C Version C.03.00 software.

LC Analyses: The HP 1090 was, in most applications, operated at 0.3 ml/min using a gradient of 25 mM Ammonium Acetate:Methanol running from 5% to 20% methanol over 20 minutes. The column was a Zorbax 2.1 X 150 mm C18, 0.5 u particle size. Also used was a 4.6 mm X 150 mm, 5 u particle size C18 column. 5 ul was the usual injection volume.

DAD Signals: For the diodarray detector, aquisition was normally made at 265 and 240 nm simultaneously.

Extraction: The extraction procedure was simplified from that described in the Agriculture Canada SUL-SPO3 method and merely uses a thorough blending of the matrix, whether meat or milk with a mixture of acetone:chloroform, 10:90 after which the organic layer is separated from the aqueous and the former layer dried down on a rotary evaporator and the residue taken up in 5 ml hexane and 2 ml of 25 mM ammonium acetate. This solution is vortex mixed, centrifuged at 2000 rpm for 1 minute and the hexane layer removed. The aqueous layer is then back-extracted with 3 ml dichloromethane. The DCM layer is then removed, taken to dryness under a stream of nitrogen, then taken up in 0.5 or 1 ml of 90:10 methanol:ammonium acetate. In some cases an internal standard (sulfachlorpyridazine) was added at an appropriate (usually 400 ppb) concentration to check recoveries. For meat samples, normally 5 grams of sample is weighed out and used in the extraction and for milk, 15 ml.

RESULTS AND DISCUSSION

LC/DAD

As stated in the introduction, our initial aim was to develop a simple LC method for the detection of sulfonamides in meat and milk. This was accomplished in a relatively short time using an isocratic LC system, but was limited to only 5 different sulfas, including the internal standard. Having seen the ease of an LC application to sulfa drug analysis we decided to go a few steps

further to look at even more sulfas using gradient LC and to introduce some rigorous confirmation protocols using the DAD and the mass spectrometer.

The gradient LC procedure we devised produced reasonably good separations of 10 different sulfas in a 20 min run, this is shown in Fig. 1. For this method we added a 5 min. re-equillibriation time making the run time a total of 25 minutes. The separation achieved in this chromatogram allows accurate identification and quantitation of the 10 sulfonamides. For each different compound a UV spectral analysis was made and the data stored in a spectral library, allowing identification of unknowns from sample runs. Data from the spectral library is shown in Figs.2(a-c) and Table 1 for sulfamethazine. These data were collected at a 10 ppm concentration of the standard. Spectra change slightly with changing concentrations so its best to collect spectra in a standard curve-like fashion. The specral library is very useful when confirming the identity of a peak above and beyond the retention time as in classic UV chromatography.

LC/MS

To achieve even more accurate data than that allowed with the DAD we introduced the samples into the mass spectrometer. Since the ionization method achieved with elecrospray MS is one of soft ionization, one has to re-think their MS theory as compared to classical electron impact MS. In the soft ionization technique, ions are generated from a nebulized spray of solvent-sample mixture. This spray, is sucked through a capillary tube into the MS source where it is exposed to a relatively high potential difference to what it experienced previously. The ionization due to this potential difference is suffficient to generate only two to three fragments along with the parent ion. Due to this comparatively light fragmentation, it is important to have the compound in as unstable a state as possible before entering the MS source. To achieve this we used an ammonium acetate buffer with only a small, gradual, increase in the concentration of methanol; the pH of this buffer allows protonation of the basic sulfa drugs and thus generation of positive ions in the MS. Since one cannot search the ion spectra produced from this fragmentation in a library, such as the Wiley, it is necessary to make-up a user library if this is desired. In the study reported here data were collected primarily in selected ion monitoring mode (SIM), which provides greater sensitivity.

Coming up with appropriate quailfier ions was probably the most difficult task in this procedure. All sulfa compounds produce the same qualifiers at 186 and 156 amu from the benzo-amine and sulfur dioxide groups respectively. However in the current study we noticed that each sulfa compound had at least two ions peculiar to the individual compound at amu higher than the parent ion and therefore we chose these ions as qualifiers. In all cases of each of the 10 sulfonamides looked at, the two qualifiers were 22 and 38 amu above the parent ion, which itself, is 1 amu above the molecular weight due to the protonation. Although the nature of these ions is unclear at the time of writing we assume they are adduct ions formed through the association of sodium and potassium, confusion occurs when one tries to figure out where the Na and K ions come from, since they are not in the running solvent or any of the injected mixtures. These adduct ions are shown in Figure 4. As a result of this finding the adduct ions were put into the SIM aquistion table as qualifiers thus greatly improving compound identification and senitivity.

At this point in the study a spiking experiment was attempted. Since no incurred meat samples were available we decided to use milk. A 15 ml milk sample was therefore spiked with 5 ppm of the mixture of 10 sulfas. This was then put through the extraction procedure outlined above and 5 ul injected onto the system. Figure 2 (a-c) shows the results of this experiment, in terms of the UV/DAD data where sulfamethazine can be confirmed using the UV spectral library search. Table 1 shows the quality of the "hit" for this search. Figure 5 shows the SIM mass spectral data for sulfamethazine from the same spiking experiment, showing the peak identification by the target or parent ion as well as the two qualifiers.

To obtain some idea of detection limits for this procedure the spiked sample was diluted to approximately 1 ppm and run on the mass spectrometer. Figures 6 and 7 show the extracted ion chromatogram and the resulting mass spectrum from this sample, respectively. Figures 8 and 9 show the same thing for a 200 ppb sample, respectively. In both cases, the compound (sulfamethazine) can be easily identified by the large parent ion and to some extent by the qualifiers. Figure 10 shows the extracted ion chromatograms for each of the 10 sulfonamides in the spiked sample.

CONCLUSIONS

From the preliminary data reported here it appears that LC/DAD/MS will prove to be a very useful tool for the analysis of relatively small molecular weight compounds present in trace amounts in various foods and enviromental samples. Detection of low ppb levels was achieved in this study and the system is still far from running at optimum. For example, the capillary line running from the DAD to the ion source of the mass spectrometer consisted of roughly three feet of 0.01 id PEEK tubing, this would have the effect of broading the peaks and reducing sensitivity. In addition the instrument was tuned on the molecular ions of three different sulfas, namely sulfanalamide, sulfamethazine and sulfadimethoxine to give a low, medium and high molecular weight range. This, however, may not be the optimum tuning parameters required, it may be better to tune on all ten sulfas. Furthermore a Cap Ex voltage of 120 was used in all the experiments outlined above. Decreasing this voltage, increases the size of the parent ion and increasing this voltage increases the size of the fragment ions. That is, the more voltage, the more fragmentation. Optimizing the Cap Ex voltage can greatly alter the results and increase or decrease sensitivity.

ACKNOWLEDGEMENT

The author would like to acknowledge the technical assistance of Jun Xia, MSc. and Li Hwang, Msc.

Figure 1: Gradient LC of 10 Sulfonamides Using DAD

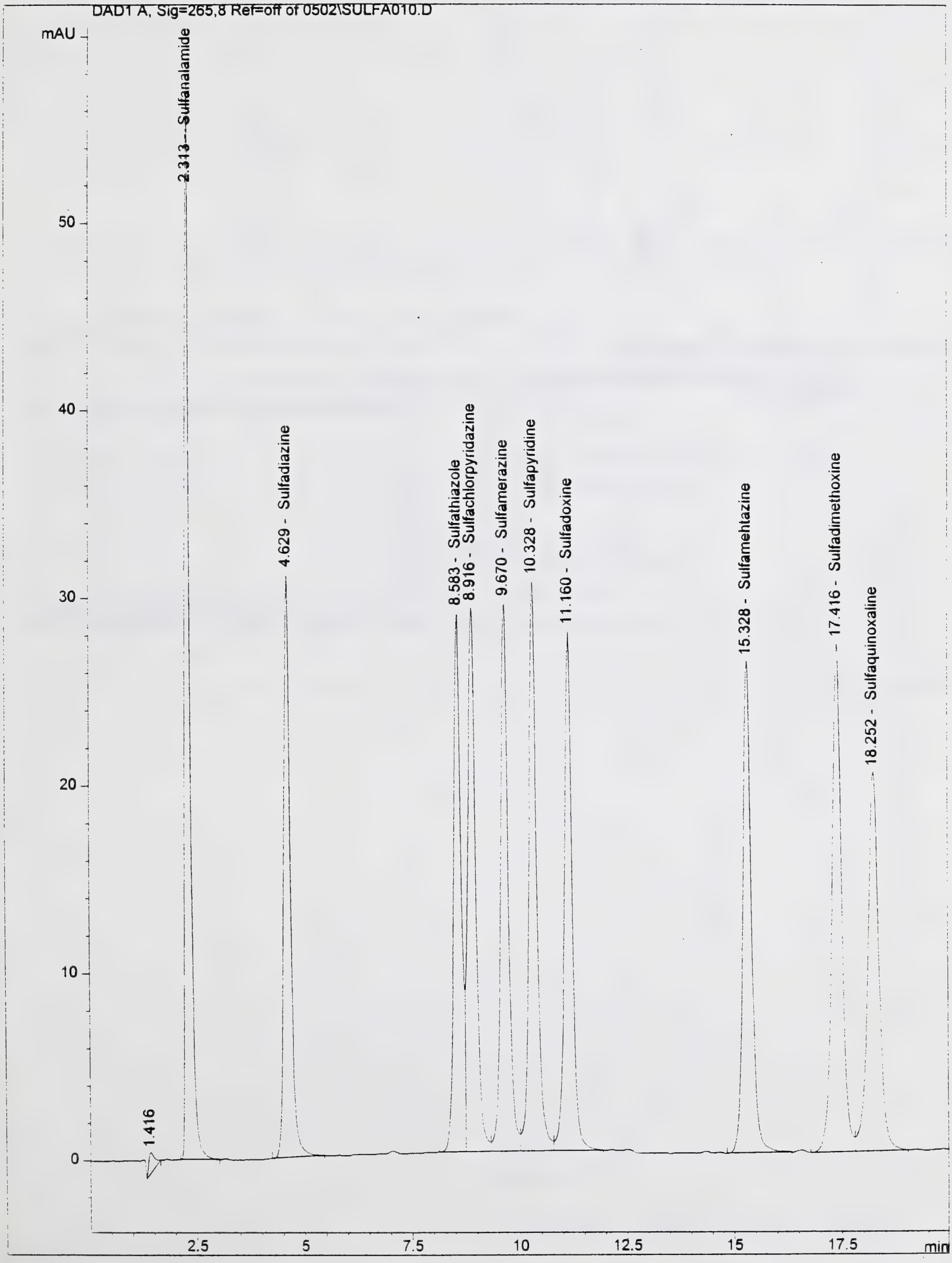


Figure 2(a-c): LC Chromatogram and Spectral Confirmation of Sulfamethazine

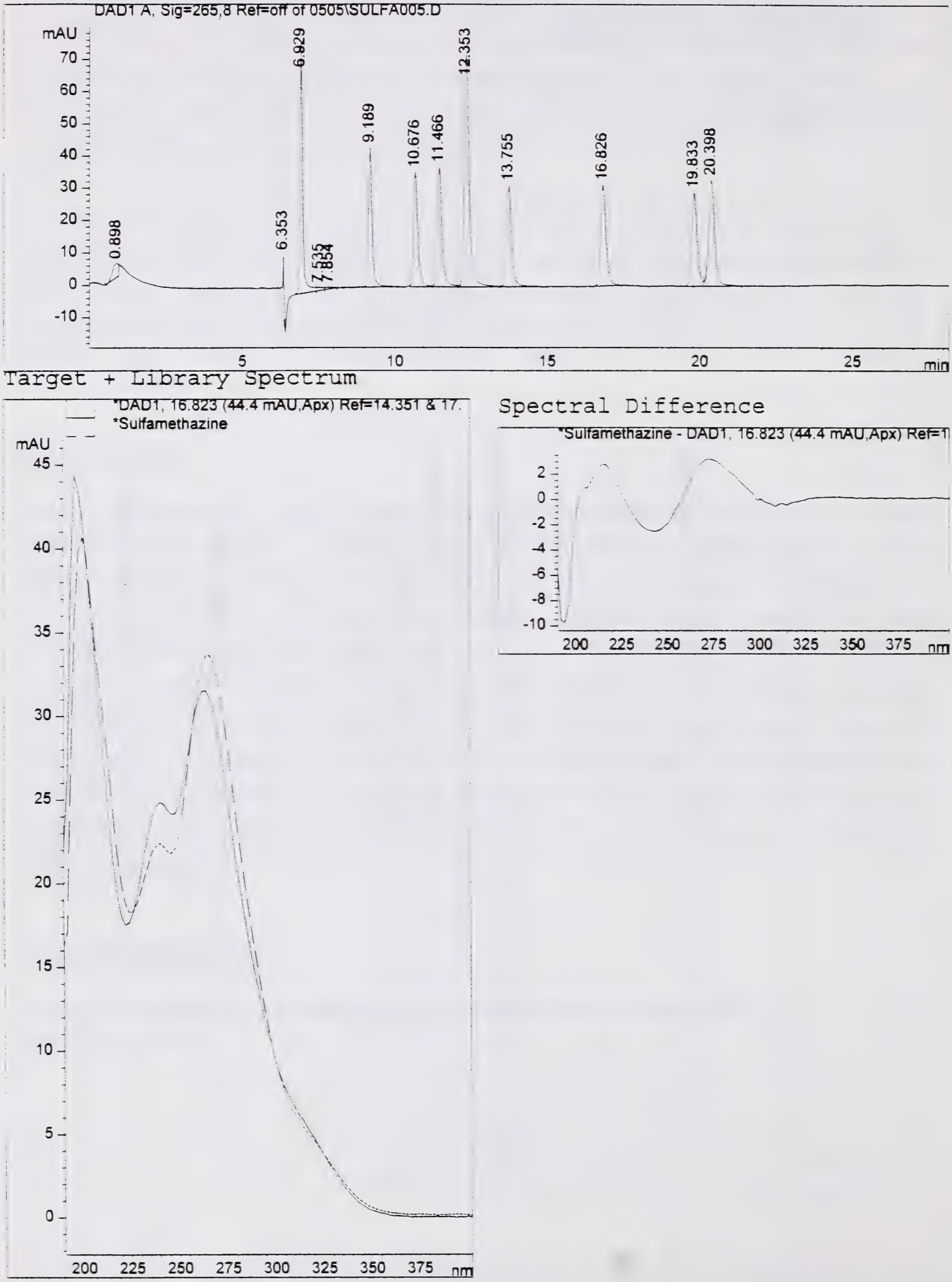


Table 1: Library Search Results for Sulfamethazine From DAD

Library search results

#	Match	Entry	Time [min]	Name
1	966.7225	3	4.911	Sulfamethazine
2	940.4525	12	4.626	Sulfadiazine
3	903.0106	2	4.005	Sulfapyridine
4	893.2520	4	5.166	Sulfachloropyridazine (IST)
5	884.5579	9	14.701	Sulfamethazine 12
6	837.1980	10	15.017	Instd 12
7	832.2369	8	3.172	sulfanilamide
8	778.2539	7	7.766	Sulfadimethoxine
9	775.2205	5	5.727	Sulfadoxine
10	732.1356	6	7.338	Sulfaquinoxaline

Figure 4: Spectrum of Sulfamethazine Showing Adduct Ions

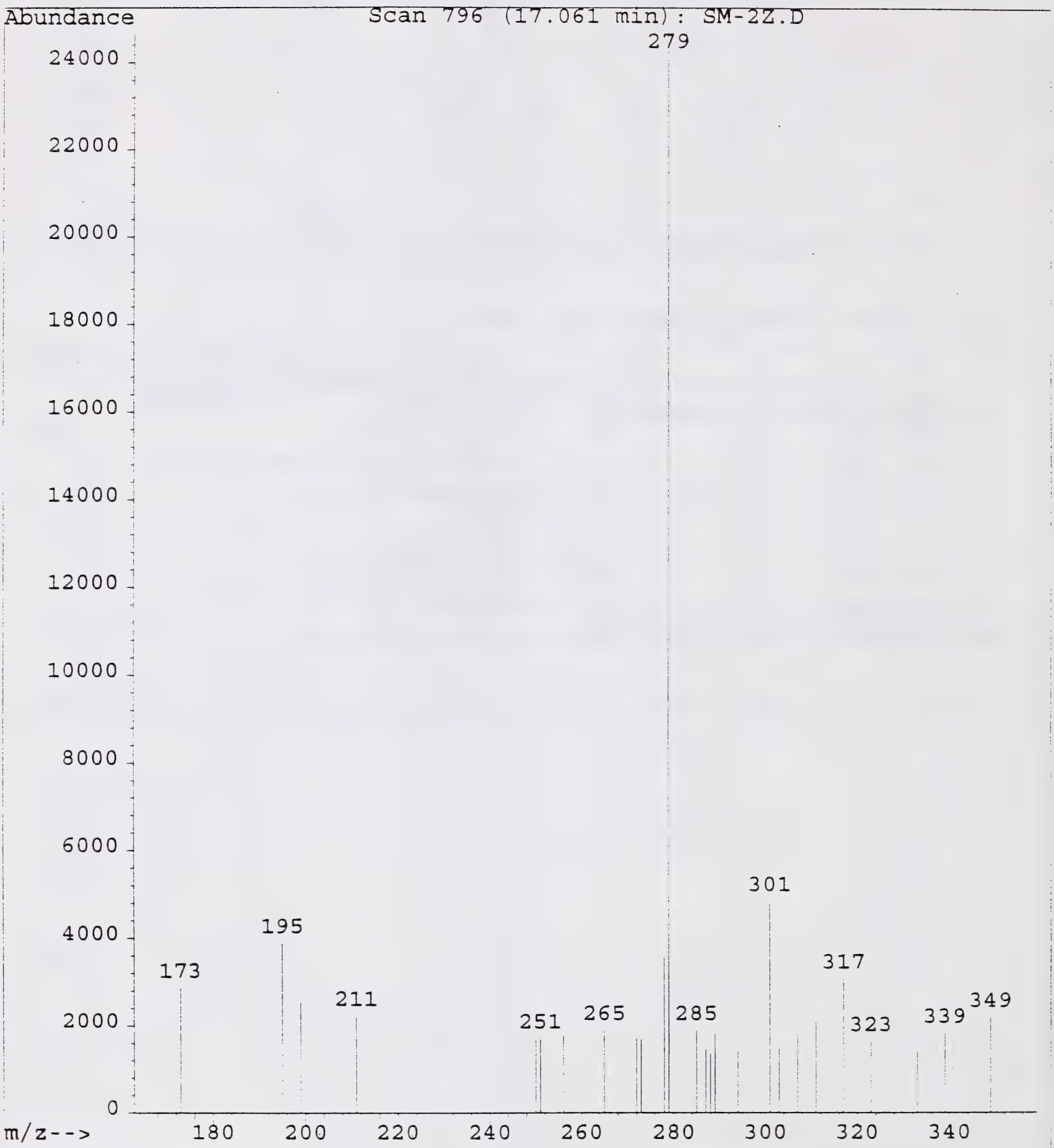
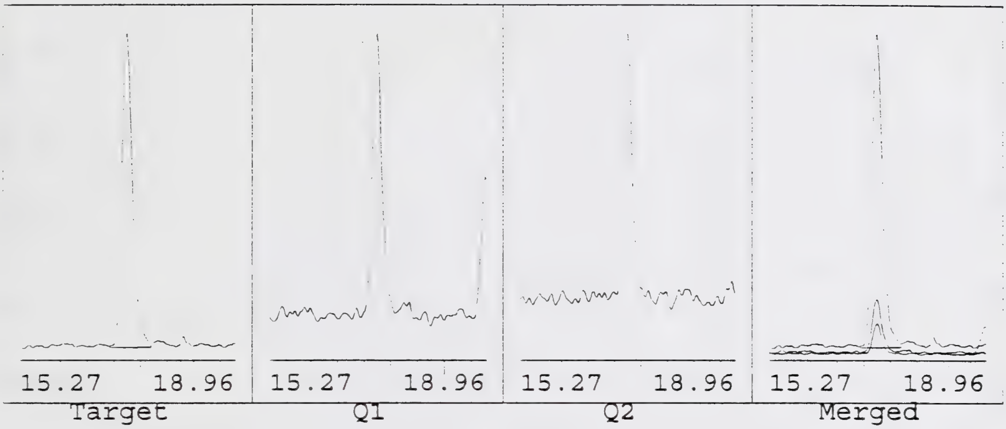
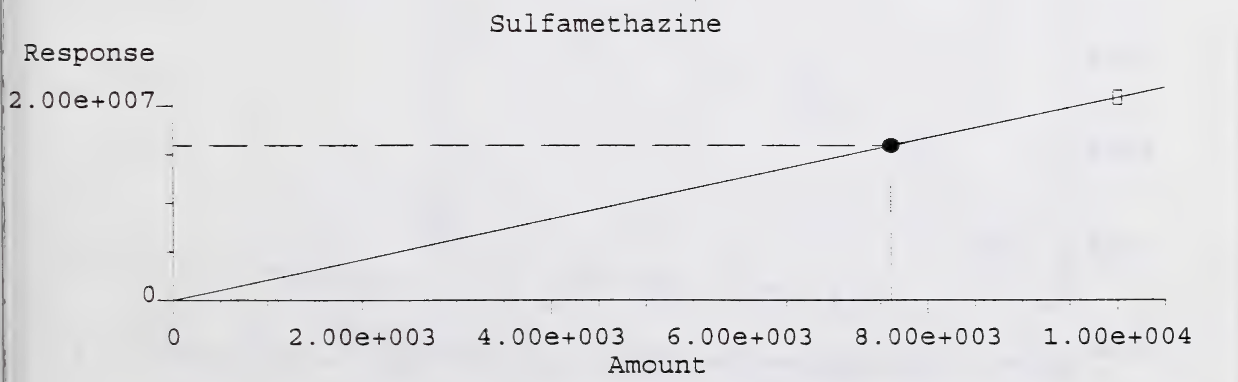


Figure 5 (a-c): SIM Mass Spectral Data for Sulfamethazine

```
Compound:          Sulfamethazine
Ret Time:          17.11
Concentration:     7610.69
Pk # and Type:     8
```



	Signal	Ratios	Limits	RT	Limits	Resp	Integ Type
gt	279.00	100.0%		17.11	16.26	15911410	e1
1	301.00	15.4	1.1- 61.1	17.12	to	2443558	e1
2	317.00	7.8	0.0- 37.9	17.13	17.97	1238727	e1
3	0.00	0.0	0.0- 0.0	0.00		0	auto



Response = 2.09e+003 * Amt + 0.00e+000
Coef of Det (r^2) = 1.000   Curve Fit: Linear

Figure 6: Extracted Ion Chromatogram for 1ppm Sulfamethazine

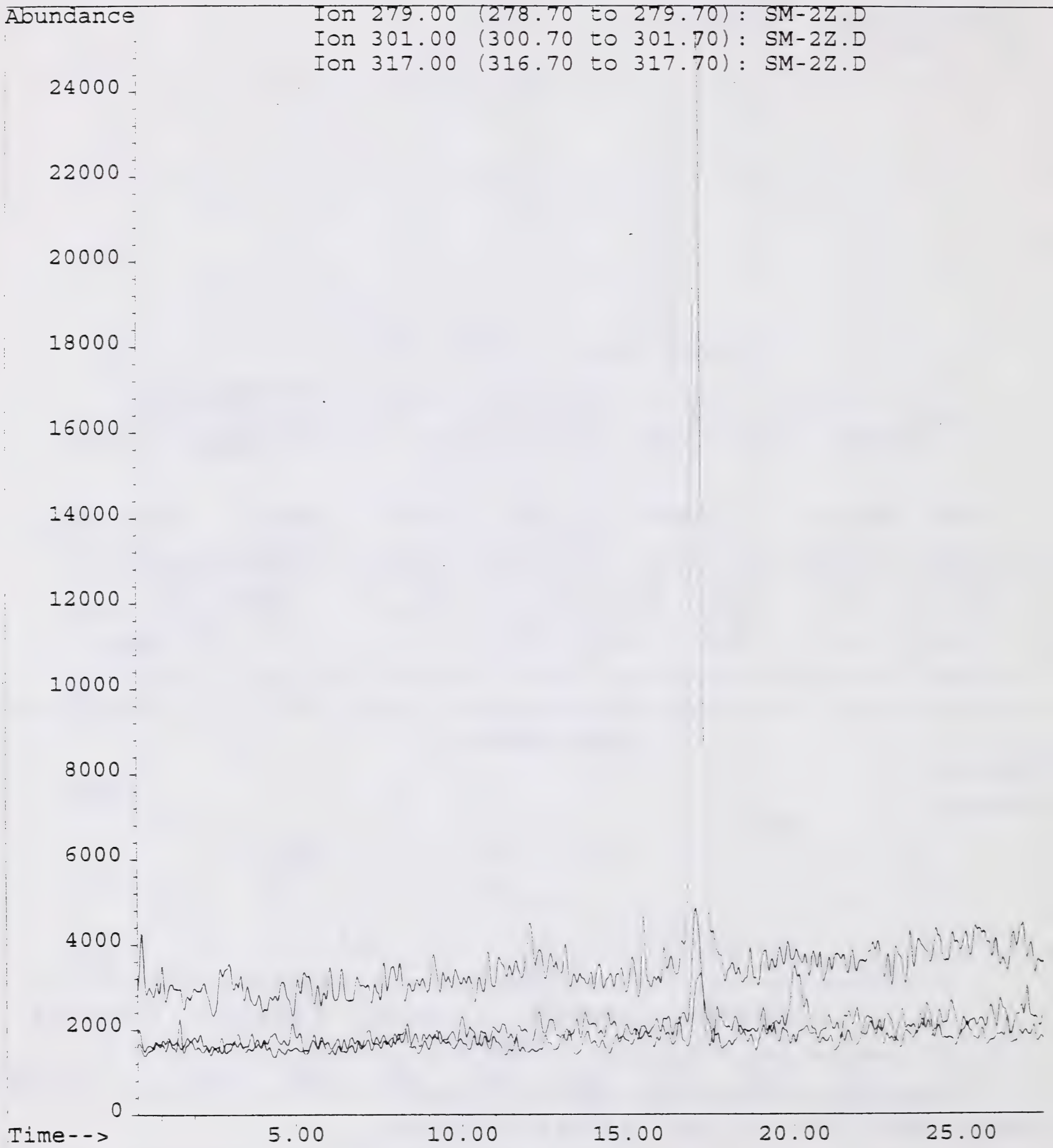


Figure 7: Mass Spectrum for 1ppm Sulfamethazine

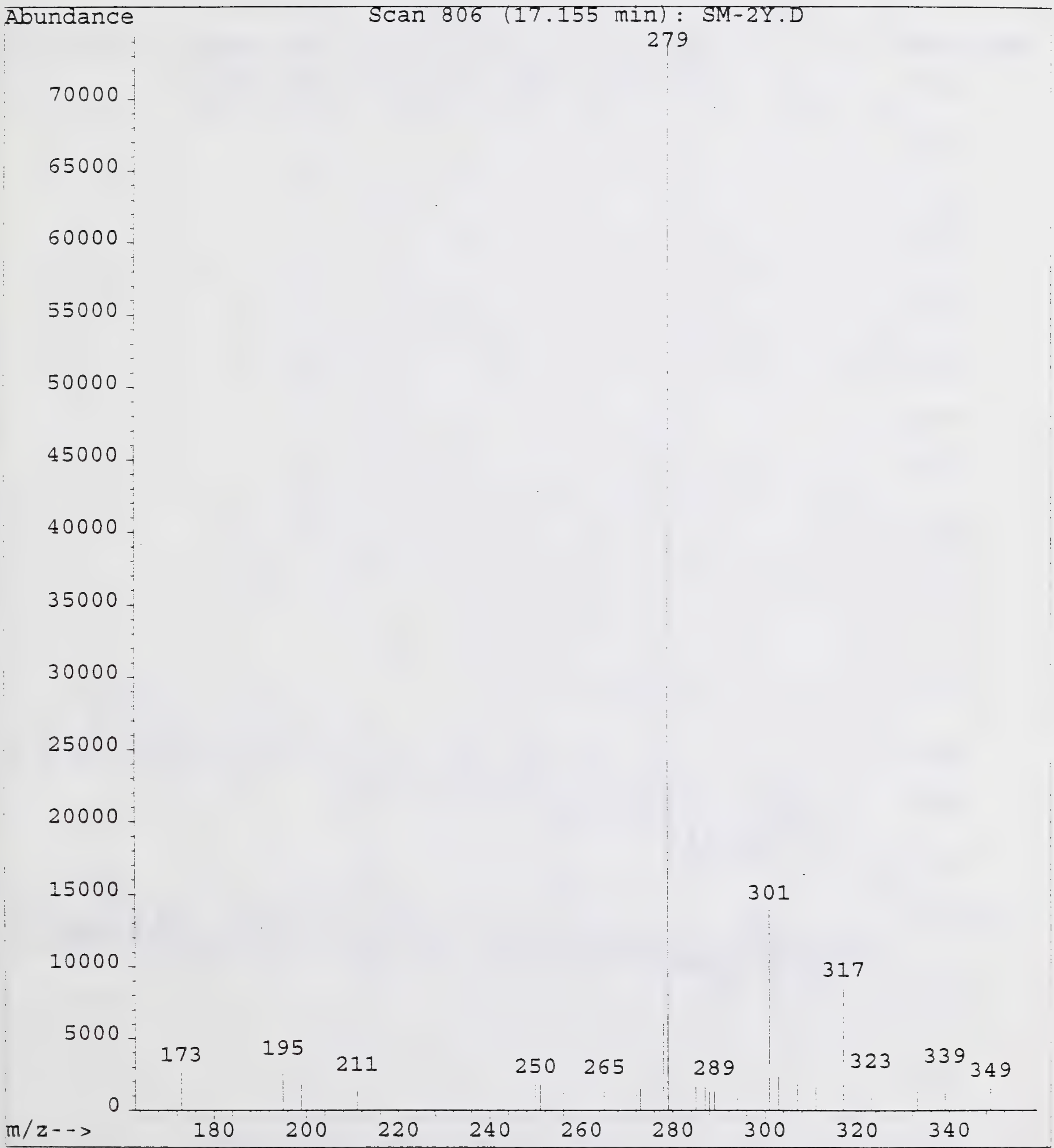


Figure 8: Extracted Ion Chromatogram for 200ppb Sulfamethazine

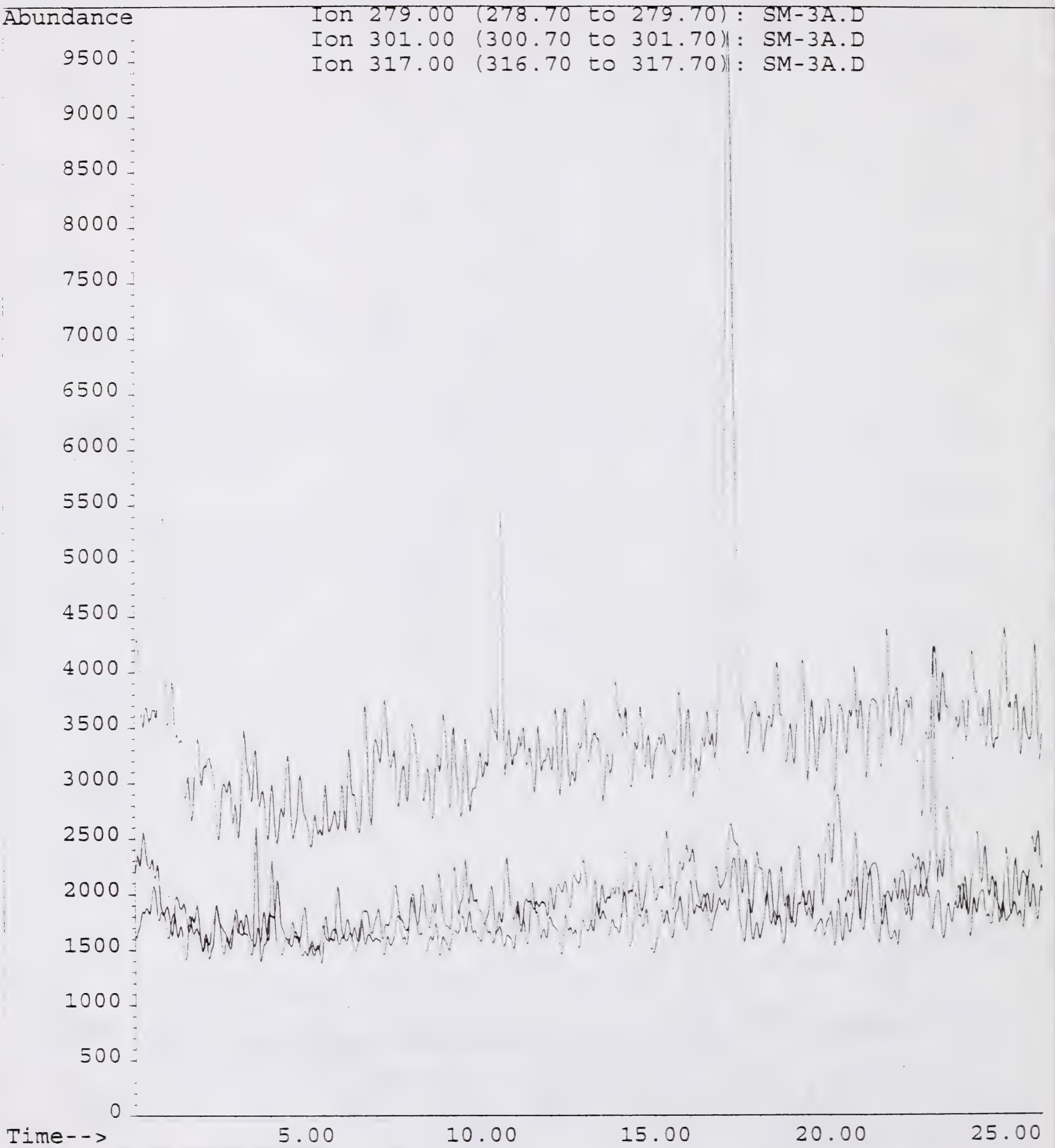


Figure 9: Mass Spectrum for 200ppb Sulfamethazine

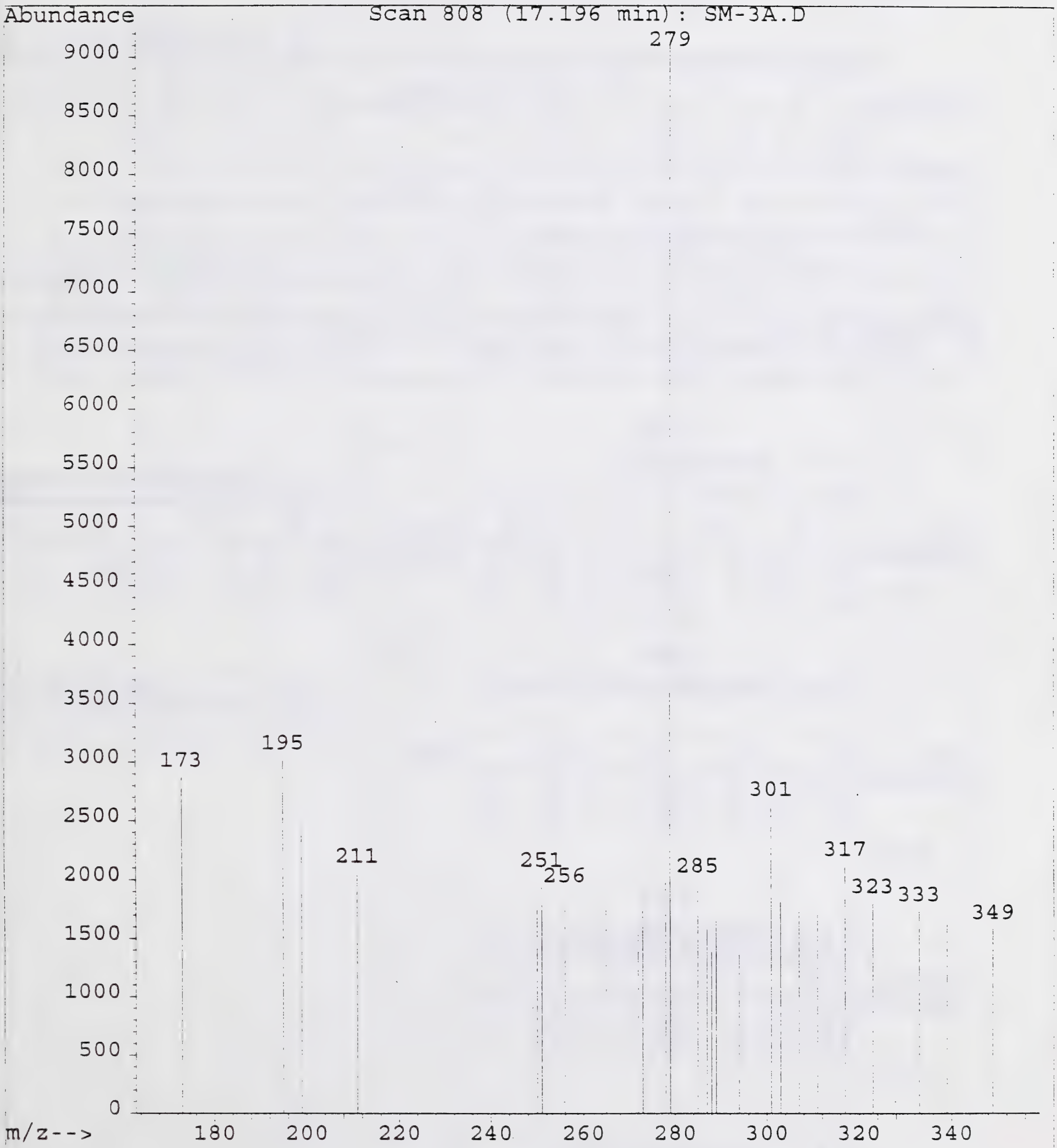
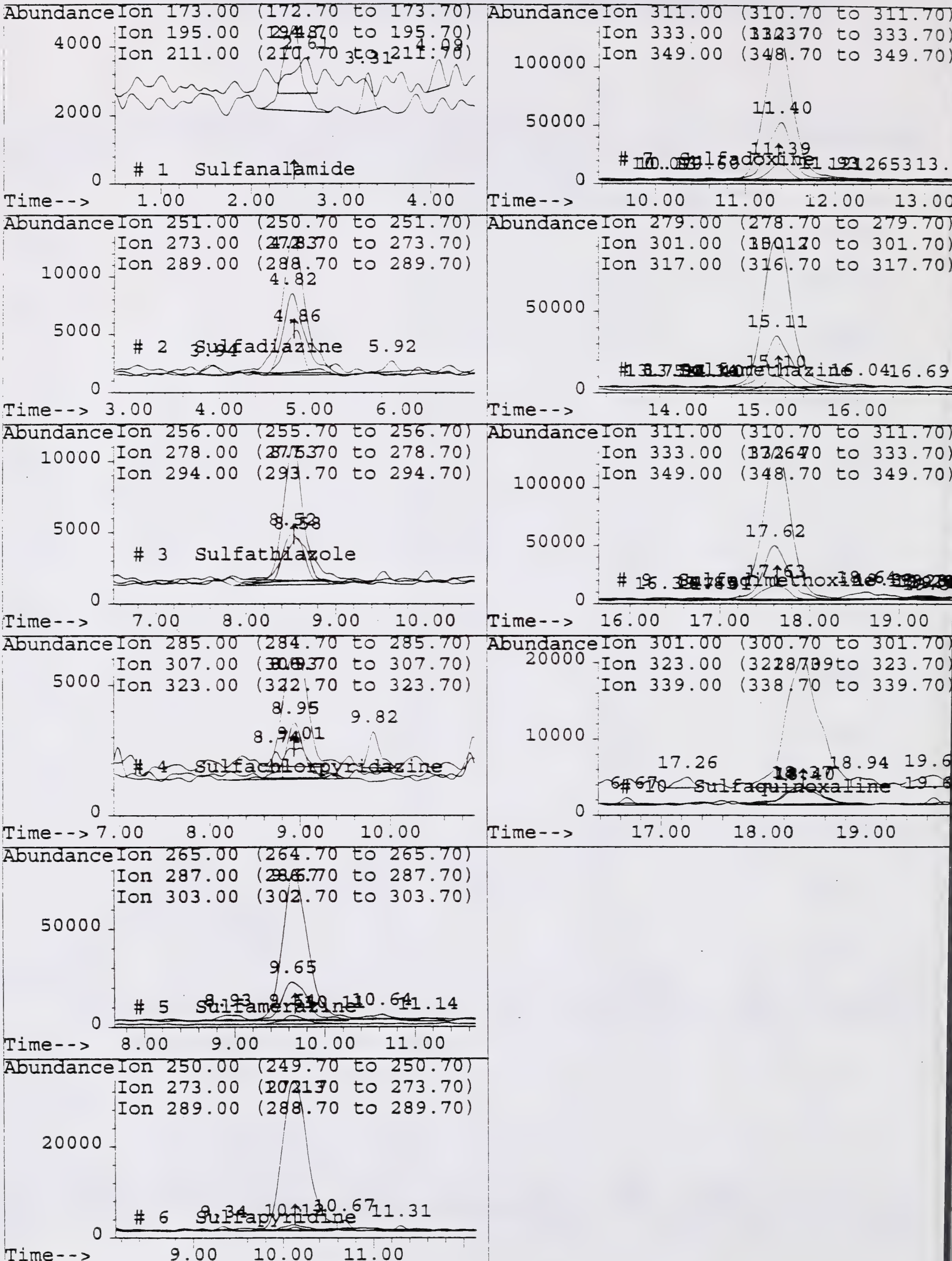


Figure 10: Extracted Ion Chromatograms for 10 Sulfonamides



DETERMINATION OF POLYCHLORINATED DIBENZODIOXINS AND DIBENZOFURNAS IN INCINERATOR FLUE GAS USING A MASS SELECTIVE DETECTOR - METHOD DEVELOPMENT AND QUALITY ASSURANCE DATA

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This paper presents the analytical methodology that has been developed and adapted, in support of the Alberta Environmental Protection and Enhancement Act, and is used to analyze, identify and quantify trace amounts of PCDD and PCDF in incinerator flue gas. Sampling, extraction and clean-up protocols for the determination of PCDD and PCDF will be described in some detail. The final extract is analyzed using GC/MSD. All measurements are performed in SIM mode. Identification of the compounds is confirmed by using target ions in correct abundance ratios. The instrumental analytical conditions and the relevant quality control data are presented. The quality assurance details including the use of isotopically labeled surrogates are discussed.

CONFIGURATION AND PERFORMANCE OF NEXT GENERATION ION TRAP MASS SPECTROMETERS

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The release of the GCQ by Finnigan MAT in January of this year marks the beginning of commercial instrumentation with the ability to tap the potential of the Ion Trap. The unique hardware configuration of the new system will be compared to current "classical" instrumentation with MS and MS/MS performance discussed. The importance of external ion generation will be discussed in some detail.

EXPERIENCES IN SET-UP OF ANALYTICAL CAPABILITY FOR CERTIFICATION OF GRAIN SHIPMENTS FOR PESTICIDE RESIDUES

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On April 1, 1994, the Canadian Grain Commission commenced pesticide residue certification of all cargo shipments of Canadian wheat and barley destined for Japan. This paper describes some of the details of this program, the major challenges faced by the Commission in instituting this program and the analytical approach taken by the Grain Research Laboratory for the compounds covered.

The Japanese Pesticide Residue Cargo Certification Program was setup in response to demands from Japanese grain importers. The agreement between the Japanese Grain Importers Association, the Canadian Wheat Board (CWB) and the Canadian Grain Commission (CGC) calls for all testing to be carried out by the CGC's Grain Research Laboratory (GRL) on a fee-for-service basis.

The GRL is the scientific arm of the CGC. The CGC is a special operating agency reporting to the Canadian Government through the Minister of Agriculture and Agri-Food. While the CGC's Industry Services Division has offices across Canada to serve the grain trade, the GRL is located at the CGC's headquarters in Winnipeg, Manitoba.

One of the Commissions major responsibilities is to ensure consistent and reliable shipments that meet contract specifications for quality, safety and quantity. The GRL plays a lead role in administering the CGC program for quality assurance of Canadian grain for toxic substances.

The GRL is currently organised under 13 sections and has a permanent staff of 110. The Residue Analysis Section was instituted in 1965 and is currently

responsible for most matters relating to grain safety. The analytical capabilities of this group cover pesticide residues, mycotoxins and trace elements. The Residue Analysis Section provides analytical support to the CGC and the grain industry in five critical areas:

- Cargo monitoring
- Suspect sample analysis
- Market support (cargo certification and noncertification fee-for-service testing)
- Grading and inspection studies
- Quality assurance studies

Despite previous involvement with other pesticide residue cargo certification programs, the Japanese project was the first major certification program to be instituted at the GRL and thus presented many challenges not previously encountered.

Compound List and Japanese Tolerance Limits

The compounds covered under the Japanese pesticide residue cargo certification program include all compounds for which tolerance limits have been established in Japan for wheat and barley. Initially, the number of compounds requested totalled 46 and 38 for wheat and barley respectively. However, by the end of 1994, the number had increased to 64 and 56 respectively. Compounds currently covered are as follows:

	JAPANESE	MRL(PPM)		JAPANESE	MRL(PPM)
COMPOUND	WHEAT	BARLEY	COMPOUND	WHEAT	BARLEY
2, 4, 5 - T	ND	ND	cyfluthrin	2.0	2.0
aldicarb	0.02	0.02	cyhalothrin	0.05	0.2
amitrole	ND	ND	cyhexatin	ND	ND
bentazon	0.2	0.2	cypermethrin	0.2	0.5
bendiocarb	0.05	0.05	daminozide	ND	ND
BHC	0.2	0.2	DDT	0.2	0.2
bioresmethrin	5.0	a	deltamethrin	1.0	1.0
bitertanol	0.1	0.05	dichlofluanid	0.10	0.10
carbon disulfide	10.0	a	dichlorvos	0.2	0.2
carbon tetrachloride	50.0	a	dieldrin	ND	ND
chinomethionat	0.1	0.1	endrin	ND	ND
chlorfenvinphos	0.05	a	ethiofencarb	1.0	1.0
chlormequat	5	5	ethoprophos	0.005	0.005
chlorpropham	0.05	0.05	ethylene dibromide	0.1	0.1
chlorpyrifos	0.1	0.1	fenitrothion	10	5.0
chlorpyrifos-methyl	10.0	a	fenobucarb	0.30	0.30

COMPOUND	JAPANESE WHEAT	MRL(PPM) BARLEY	COMPOUND	JAPANESE WHEAT	MRL(PPM) BARLEY
fenvalerate	2.0	2.0	parathion	0.3	0.3
flucythrinate	0.20	0.20	parathion-methyl	1.0	1.0
flutolanil	2.0	2.00	pendimethalin	0.2	0.20
glufosinate	0.20	0.20	permethrin	2.0	2.0
glyphosate	5.0	20	phosphine	0.1	a
imazalil	0.01	0.05	phoxim	0.05	0.05
inorganic bromide	50	50	piperonyl butoxide	20.0	a
iprodione	10	10	pirimicarb	0.05	0.05
malathion	8.0	2.0	pirimiphos-methyl	1.0	1.0
mepronil	2.00	2.00	propiconazole	1.0	1.0
methiocarb	0.050	0.050	pyrethrins	3	3
methoprene	5.0	5.0	terbufos	0.01	0.01
methoxychlor	2.0	a	thiobencarb	0.1	0.1
metribuzin	0.75	0.75	thiometon	0.020	0.020
myclobutanil	0.3	0.5	trichlorfon	0.10	0.10
oxamyl	0.02	0.02	triflumizole	1.0	1.00

a No tolerance limit established under Japanese legislation
ND Not detectable

Certification was requested for all of the above compounds despite the fact that 50 of the 64 compounds for wheat and 45 of the 56 compounds for barley are not licensed in Canada for use on these grains and 39 of the 64 compounds are not licensed in Canada for use on any cereal grain, oilseed or pulse crop.

Lead Time For Implementation Of Certification Program

The GRL had from mid-November, 1993 until March 31, 1994 to complete arrangements for set-up of the certification service. Further requests to cover additional compounds were received in February, 1994 and October, 1994 and these were subsequently added in for shipments loaded as of July 1, 1994 and December 12, 1994 respectively.

Turnaround Time For Results

For 1994/95, the requested maximum turnaround time for results was 14 days (time interval between the bill of lading date and transmission of the final report to the respective grain importers).

Samples

All shipments of wheat and barley destined for Japan are loaded at terminal elevators in either Vancouver or Prince Rupert. Official CGC cargo loading samples are taken by automatic samplers. For each shipment of wheat and

barley destined for Japan, a 4 kg portion of the official cargo loading sample is split off for pesticide residue certification and subsequently forwarded to the GRL in Winnipeg by courier. On arrival at the GRL, samples are stored in a freezer until analyses commence.

Laboratory Renovations

In order to consolidate the Residue Analysis Section's GC and HPLC instrumentation and to accommodate the additional equipment which had to be purchased, a major renovation was undertaken to construct an instrument room. This required relocating another GRL group which in turn necessitated renovation of the new home for this group before renovation of the new instrument room could proceed.

Other important preparations included the establishment of a second sample preparation laboratory and creation office space for the new certification chemists.

One of the inevitable consequences of rapid expansion of the Residue Analysis Section that had to be dealt with was the unilization and sharing of bench space, fume-hood space and equipment.

Staffing

A Certification Unit was created within the Residue Analysis Section in order to deal specifically with the analytical workload of the Japanese Pesticide Residue Certification Program. This necessitated writing job descriptions and initiating competitions for the Chemist-In-Charge of this unit and the chemists that would carry out the certification analyses. To date, a total of six chemists have been hired for this unit.

Equipment Purchases

In setting up for this new program, a large number of capital equipment purchases were necessary. This included a number of major chromatography instruments and many items for sample preparation purposes including everything from a sample grinder to a muffle furnace. Being an agency of the Federal Government, lengthy purchase specifications had to be prepared for each item and we were required to follow the normal lengthy pathway for government purchases.

Given the short lead time and the necessity to have most of the new instrumentation up and running by April 1, 1994, equipment purchases had to be expedited in every possible way. Two of the ways that DSS proved helpful with respect to the chromatography instrumentation purchases was by approving sole supplier status for some of our purchases and by shortening the tender period for some others.

Shipment Documentation

An information system was setup with the CWB to provide vessel nomination details about upcoming shipments. In addition to obtaining information vital for preparation of documents and other correspondence, this also provides a means for double-checking on sample deliveries.

Documents

A computerised system linking both EXCEL spreadsheets and Microsoft Word was setup to facilitate sample documentation and tracking, recording of results and generation of the final reports and other correspondence including invoice requests and various cover letters.

Laboratory Operations

Although a separate Certification Unit was created to carryout the day-to-day analyses of the certification program, due to the lengthy training period necessary to familiarize the certification chemists with the ananlyical methods and instrumentation, this unit was not fully operational until June, 1994. During this training period, much of the cargo certification work was carried out by the Residue Analysis Section's Analytical Support Unit. This unit continues to provide the analytical support, personnel backup, troubleshooting and method development support for this program.

One of the inevitable consequences resulting from rapid expansion of the Residue Analysis Section and attempting to incorporate two major programs within the same laboratory space was utilization and sharing of bench space, fume-hood space, sample preparation equipment and chromatography instrumentation.

Other factors that affected laboratory operations included matters pertaining to safety, quality assurance and operating supplies.

Analytical Methods

A total of 16 separate methods were found to be necessary in order to cover all 64 compounds. These involved a total of 11 extraction procedures, 8 cleanup procedures, 6 derivatization procedures (not including the post-column HPLC procedures) and 10 determinative procedures.

The major contributor to the large number of methods is the large proportion of single-compound methods. While many of the compounds on the Japanese list simply do not lend themselves to multiresidue methods, there were a few compounds for which separate methods were developed despite initial expectations that we might be able to include them under other methods. With the start-up date being a major time constraint, it was sometimes necessary to short-circuit the normal method development process and develop totally separate methods for compounds for which troublesome problems were being encountered in attempting to add them into an existing method. In many cases, this proved to be the shortest route for establishing analytical capability for "problem" compounds. Reduction of the number of methods would come later.

Method	Cpds	Extraction Technique	Cleanup Technique(s)	Derivatization Technique	Determination Technique
OC/OP/ON compounds & pyrethrins	41	homogenize ground grain with DCM + acetone (1+1, v/v)	gel permeation chromatography on 50g BioBeads SX-3 column with DCM + cyclohexane (15+85, v/v)	none	GC - MSD - selected ion monitoring and GC - ECD
glyphosate	1	shake ground grain with acidified $CHCl_3$	cation ion exchange on AG 50-X8 resin/ anion ion exchange on AG 1-X8 resin	sequential post-column HPLC oxidation with calcium hypochlorite solution and derivatization with o-phthalaldehyde	ion exchange HPLC with fluorescence detection
total bromide	1	hydrolyze with alkaline monoethanolamine then ash at 500° C	none	oxidation with potassium permanganate	fluorescein impregnated Florisil detector tube
chlormequat	1	homogenize ground grain with methanol	aluminum oxide (acid) column eluted with acetone + methanol (1+1, v/v) and (95+5, v/v)	none	TLC on silica gel developed with water + acetonitrile + acetic acid (4+4+1, v/v/v) and treated with a chromogenic spray (Dragendorff's Reagent)
bitertanol	1	homogenize ground grain with DCM + acetone (1+1, v/v)	Florisil Sep-Pak cartridge eluted with ethyl acetate+hexane (1+49, v/v), (3+17, v/v) and (4+6, v/v)	trifluoroacetic anhydride	GC - MSD - selected ion monitoring
N-methyl carbamates	6	homogenize ground grain with DCM + acetone (1+1, v/v)	aminopropyl Sep-Pak cartridge eluted with methanol + DCM (1+99,v/v)	post-column HPLC derivatization with o-phthalaldehyde	reversed phase HPLC with fluorescence detection
dichlofluanid	1	homogenize ground grain with DCM + acetone (1+1, v/v)	Florisil Sep-Pak cartridge eluted with ethyl acetate + hexane (1+49, v/v) and (3+17, v/v)	none	GC - MSD - selected ion monitoring

Method	Cpds	Extraction Technique	Cleanup Technique(s)	Derivatization Technique	Determination Technique
methoprene	1	homogenize ground grain with acetonitrile	partition with hexane, cleanup on Florisil Sep-Pak eluted with hexane + ethyl ether (95+5, v/v)	none	GC - MSD - selected ion monitoring
liquid fumigants	3	grind with crushed dry- ice, heat ground grain in head space vial	none	none	headspace GC - ECD
daminozide	1	hydrolysis	distillation	react with salicylaldehyde	GC - MSD - selected ion monitoring
glufosinate	1	homogenize ground grain with water	anion exchange on Dowex 1X8-100 resin , silica gel Sep-Pak cartridge eluted with methanol + ethyl acetate (1+1, v/v)	trimethylorthoacetate/ acetic acid	GC - FPD (P)
2, 4, 5 - T & bentazon	2	homogenize ground grain with acidified isopropyl alcohol	Sephadex LH - 20 column with isopropyl alcohol eluent	diazomethane	GC - MSD - selected ion monitoring
cyhexatin	1	open vessel microwave digestion with nitric acid and hydrogen peroxide	none	none	transverse heated Zeeman GF-AA spectroscopy
amitrole	1	homogenize ground grain with methanol	Sephadex LH - 20 column with isopropyl alcohol eluent	methyl-8 - concentrate	GC - MSD - selected ion monitoring
phoxim	1	homogenize ground grain with DCM + acetone (1+1, v/v)	aminopropyl Sep-Pak cartridge eluted with DCM + methanol (99+1, v/v)	none	reversed phase HPLC - UV detection
phosphine/ AlP	1	reflux in dilute H_2SO_4 under partial vacuum	none	none (acid hydrolysis takes place during refluxing)	GC - FPD (P)

LC/MS AND LC/MS/MS ANALYSIS OF PESTICIDES IN DRINKING WATER

BY

NICK HOLLAND

FISONS INSTRUMENTS INC.

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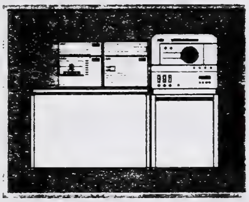

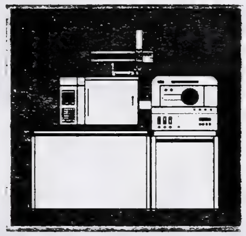

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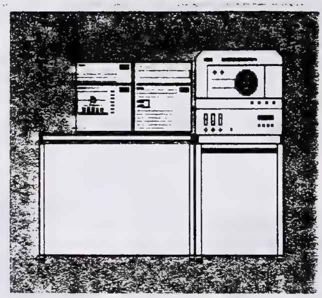

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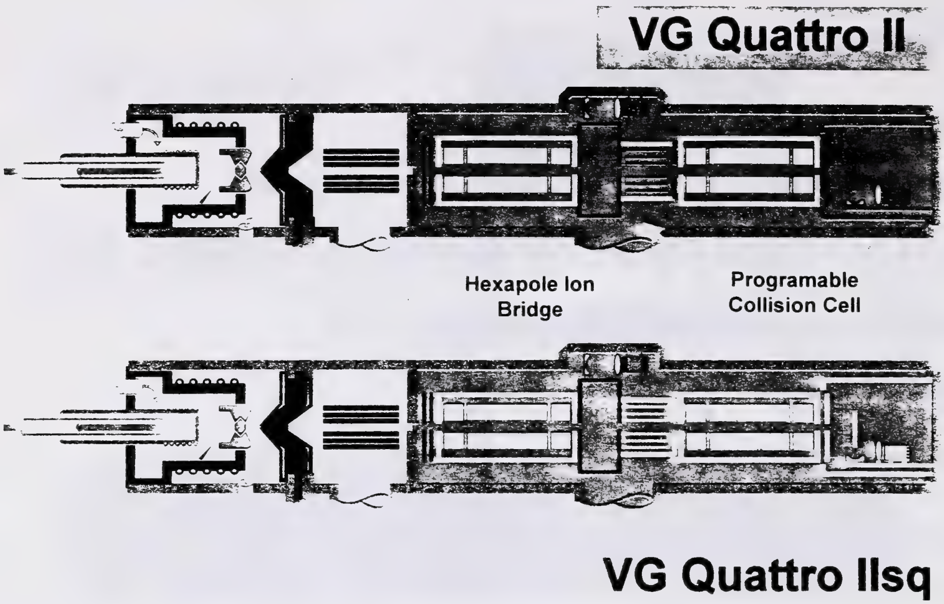

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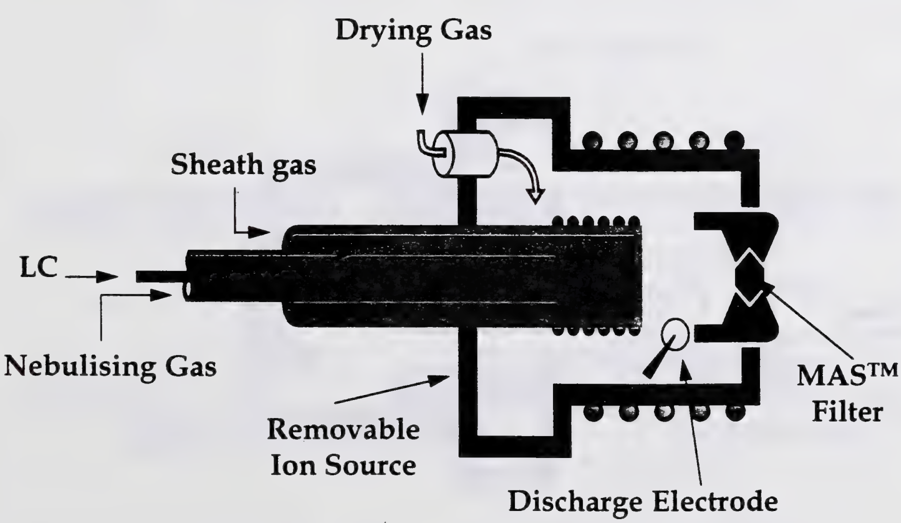

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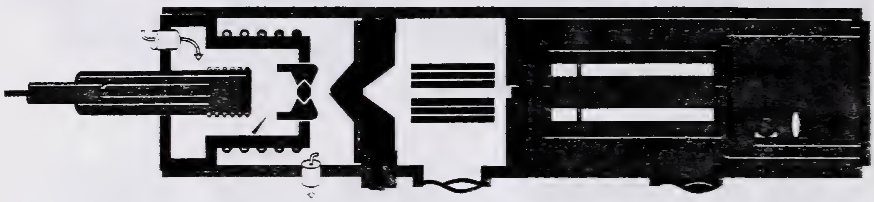
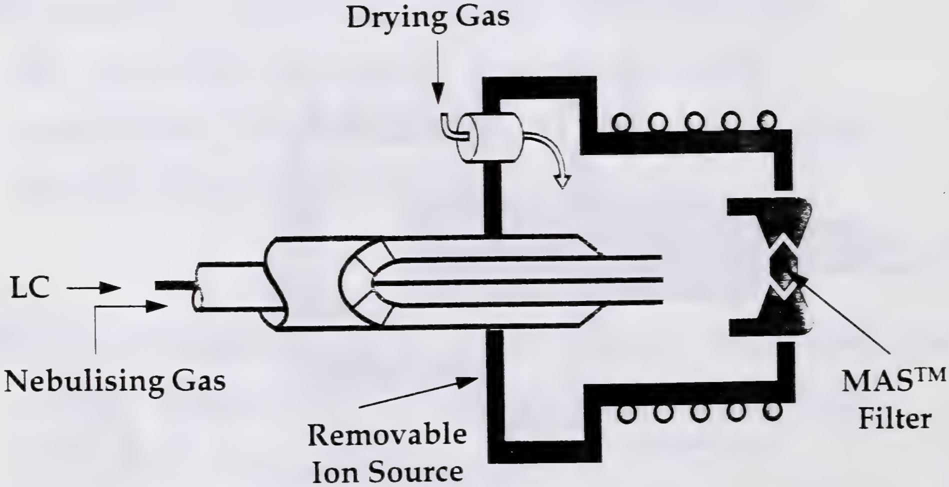
■ The mass spectrometer

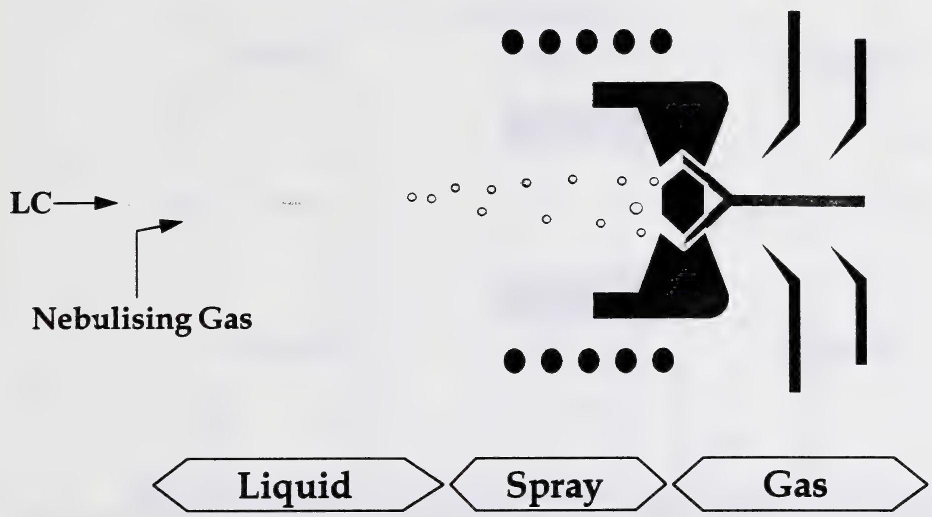


API Inlet Technology

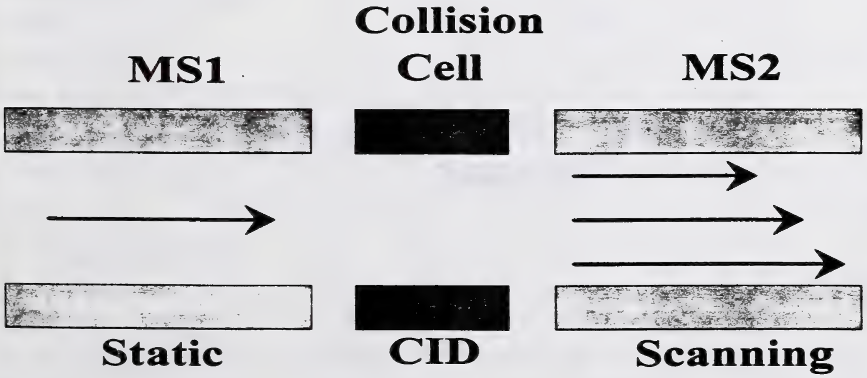


Exchangeable Megaflow ES Inlet

Megaflow Electrospray

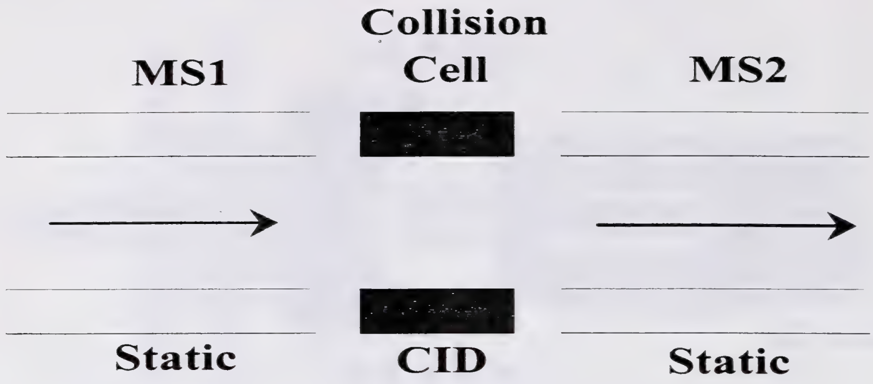


Product Ion Spectrum



MS/MS : Structural analysis

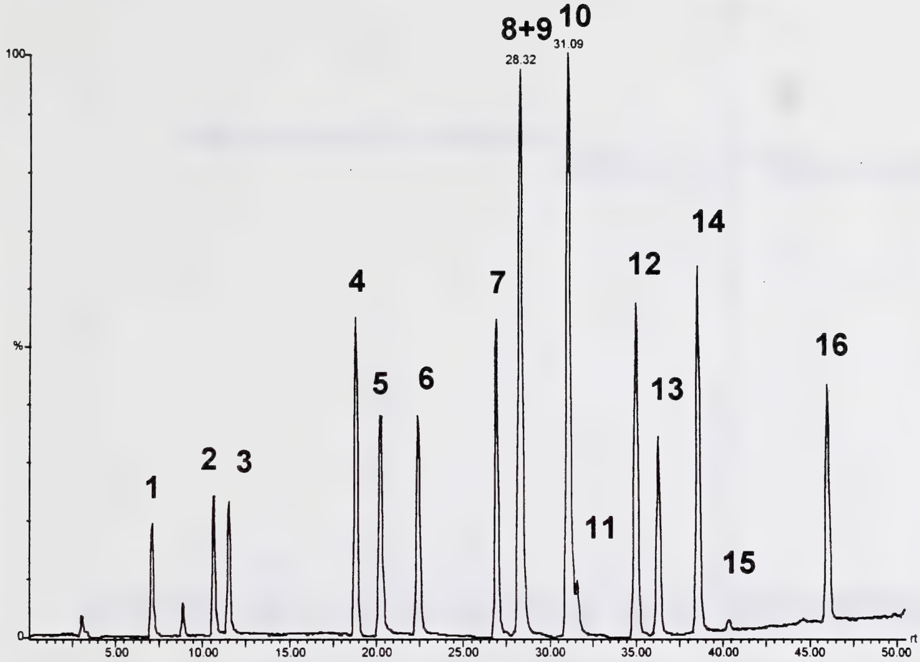
Multiple Reaction Monitoring (MRM)



MS/MS : Target compound analysis

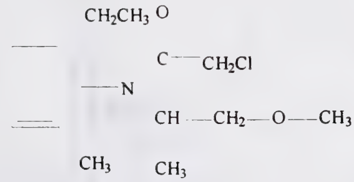
Quantitation

	Specificity	Speed	Sensitivity Clean Matrix	Sensitivity Dirty Matrix
SIR (MS)	✓	✓	✓ ✓ ✓	✓
MRM (MS/MS)	✓ ✓ ✓	✓ ✓ ✓	✓ ✓	✓ ✓ ✓

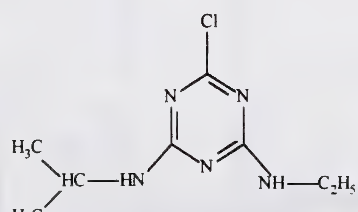


TIC from the LC/MS analysis of 16 herbicides (10ng each on-column)

1. Desisopropylatrazine
2. Metamitron
3. Desethylatrazine
4. Metoxyuron
5. Simazine
6. Cyanazine
7. Metabenzthiazuron
8. Chlorotoluron
9. Atrazine
10. Isoproturon
11. Diuron
12. Metazachlor
13. Propazine
14. Terbutylazine
15. Linuron
16. Metolachlor

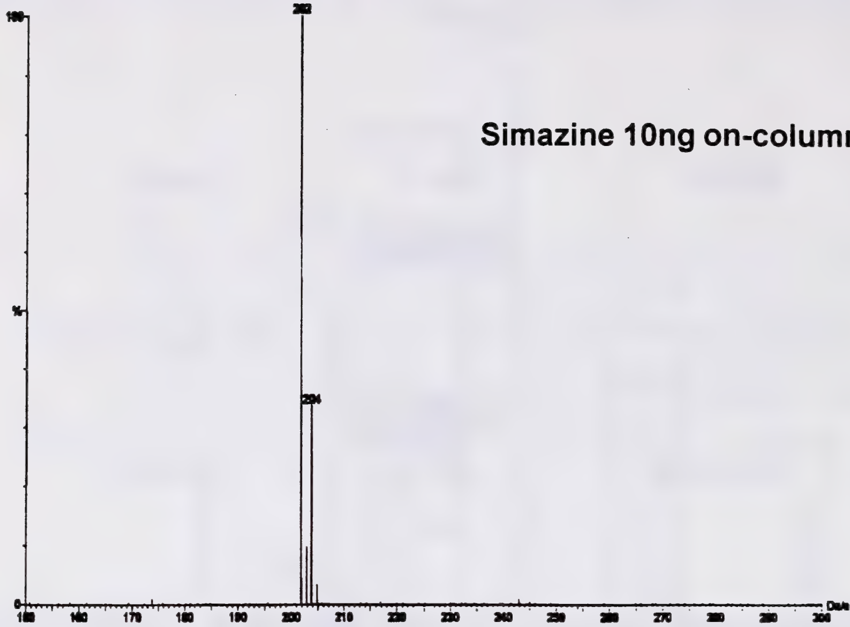


Metolachlor

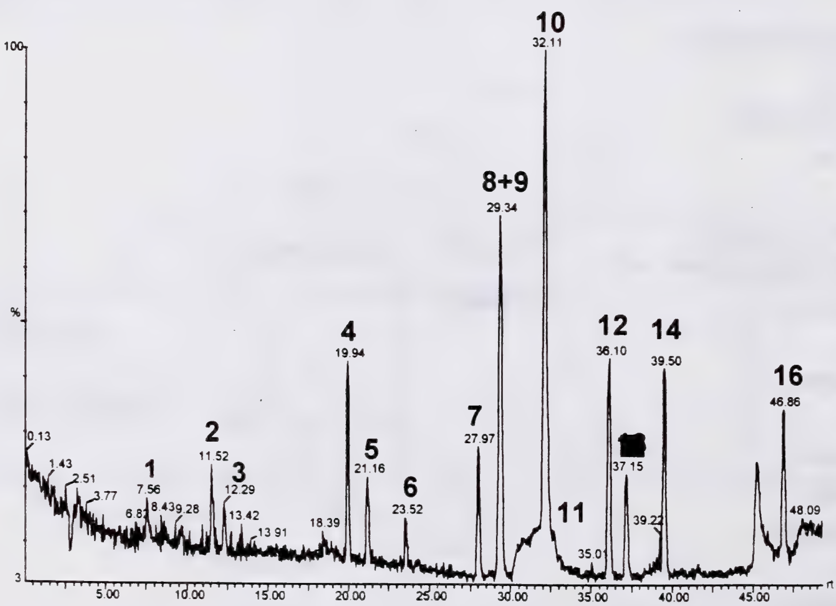


Atrazine

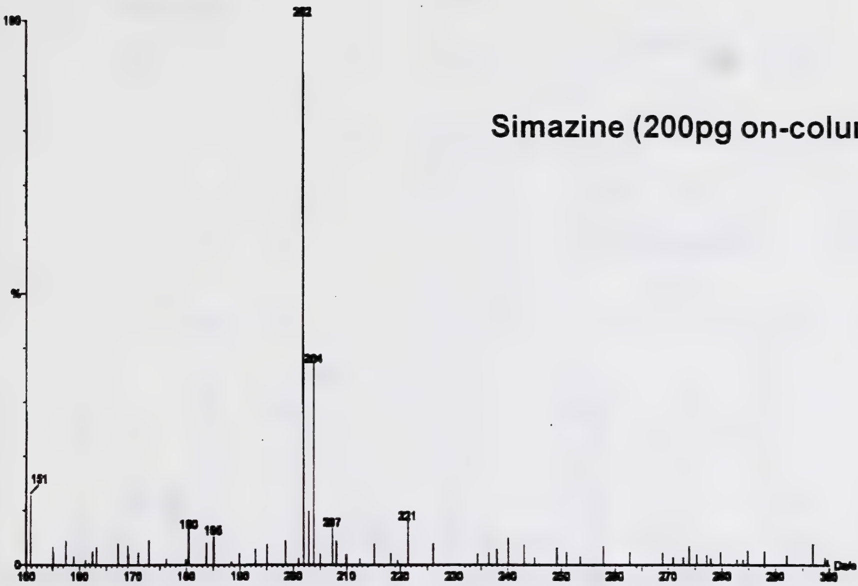
Phenylurea and triazine herbicides



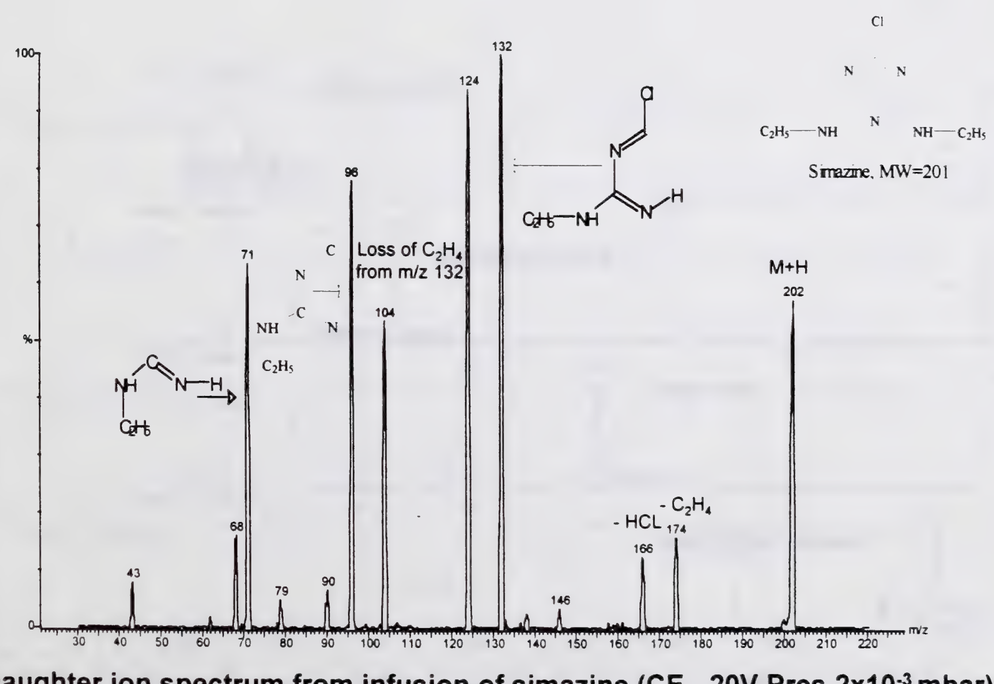
Spectrum taken from LC/MS analysis



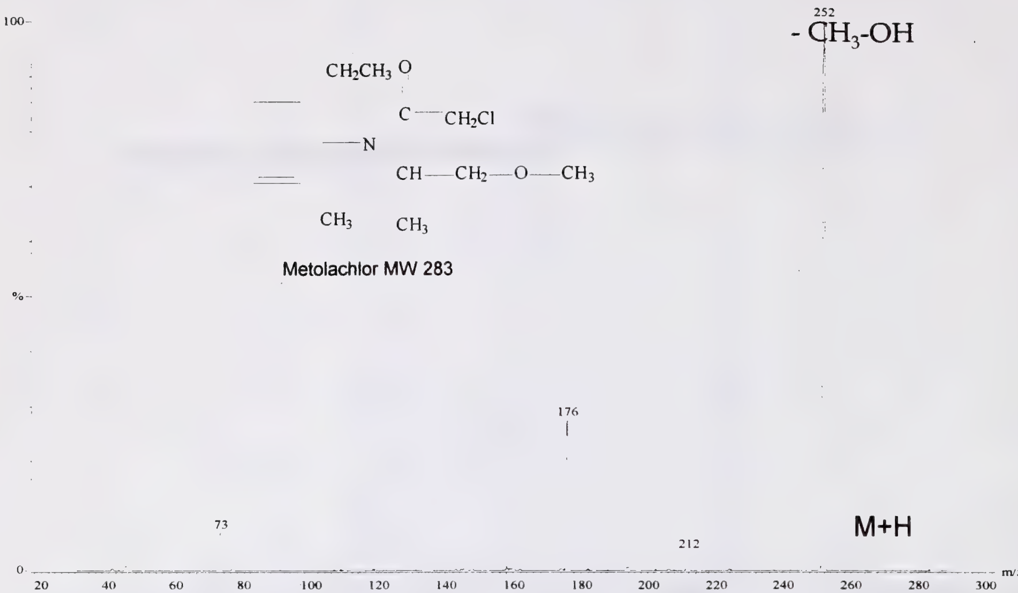
TIC from the LC/MS analysis of 16 herbicides (200pg on-column injection)



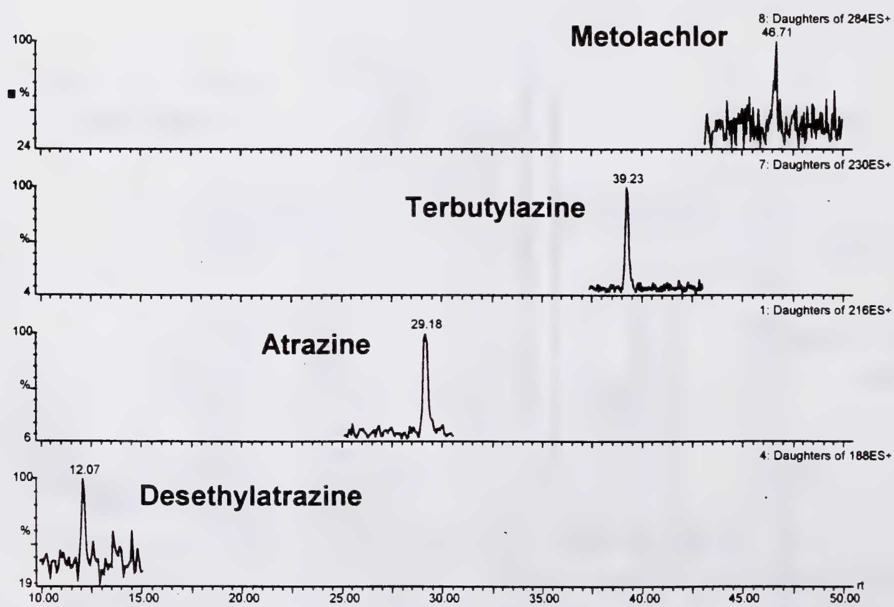
Spectrum taken from LC/MS of herbicide mix



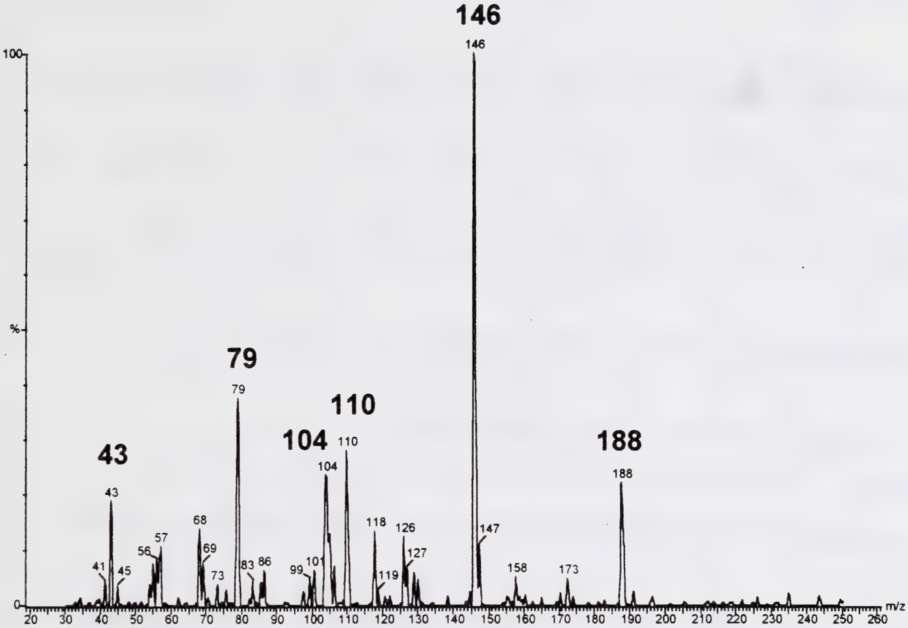
Daughter ion spectrum from infusion of simazine (CE - 20V Pres.$2x10^{-3}$ mbar)



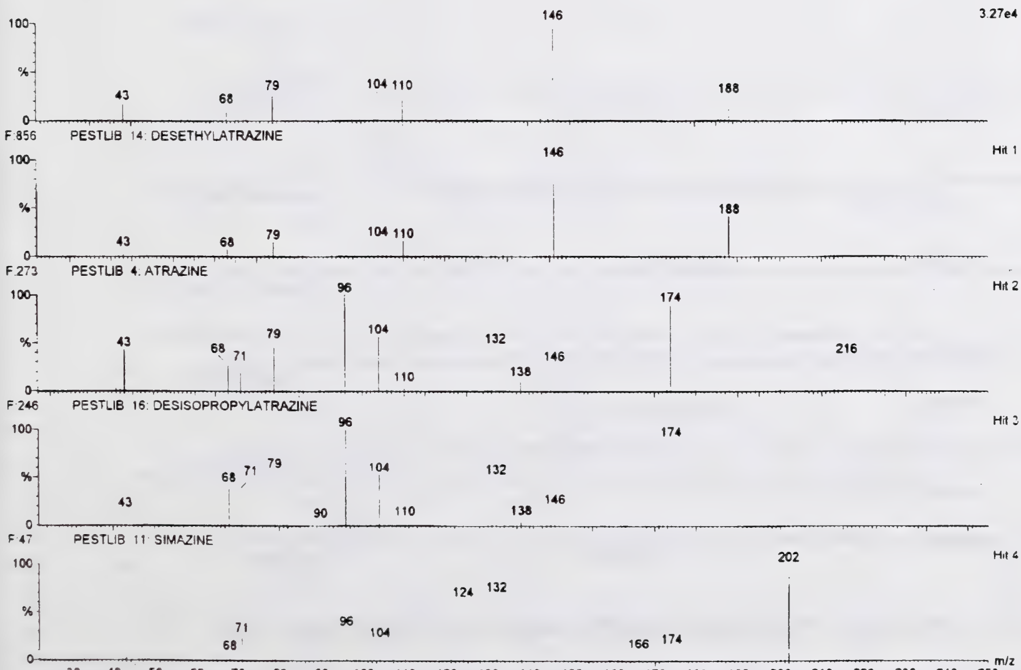
Daughter ion spectrum from infusion of metolachlor (CE =20V Pres. $2x10^{-3}$ mbar)



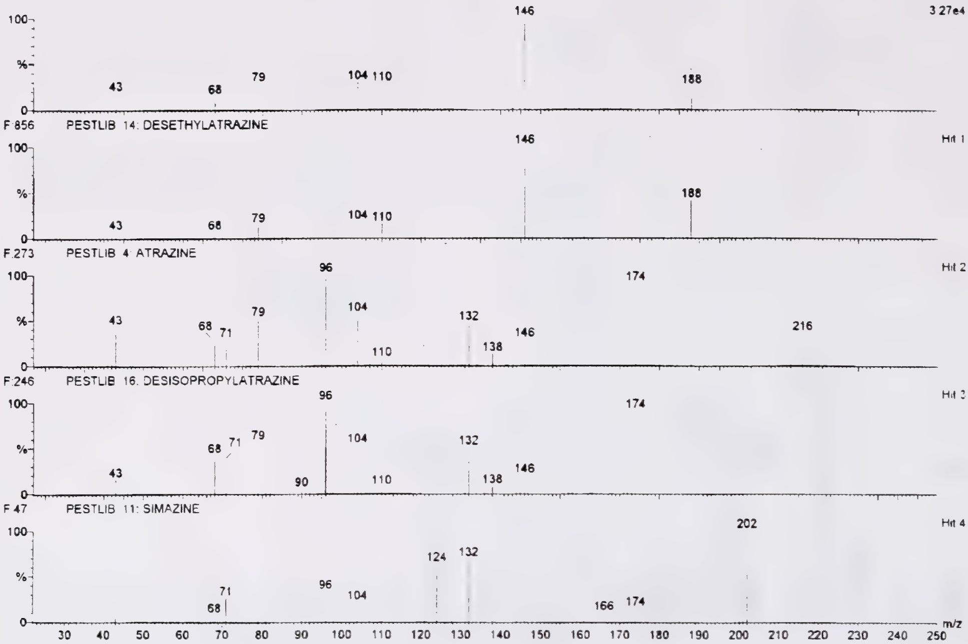
Daughter ion TICs of herbicides at 50pg on-column



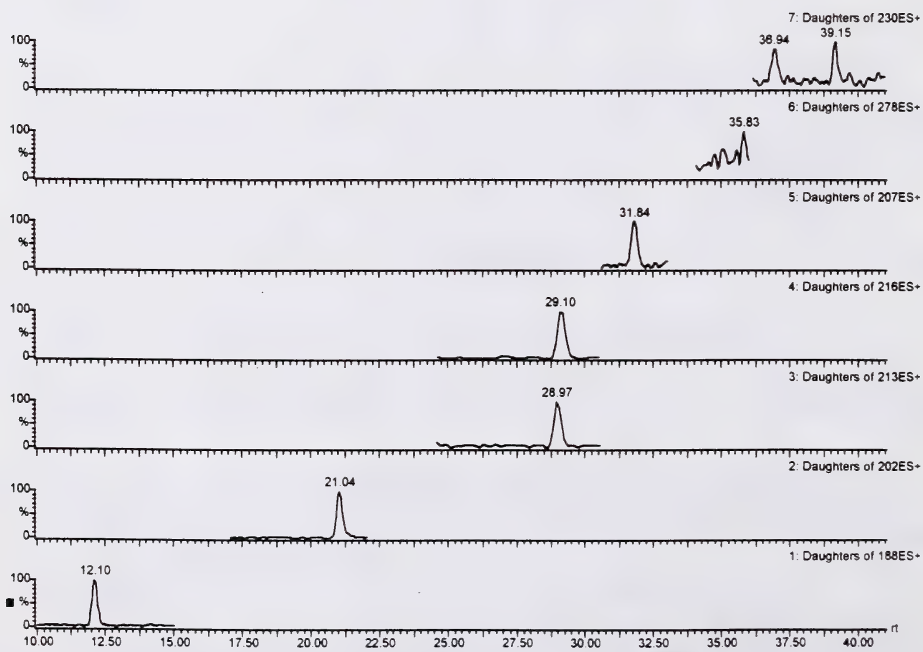
Daughter ion spectrum from LC/MS/MS analysis of desethylatrazine (50pg)



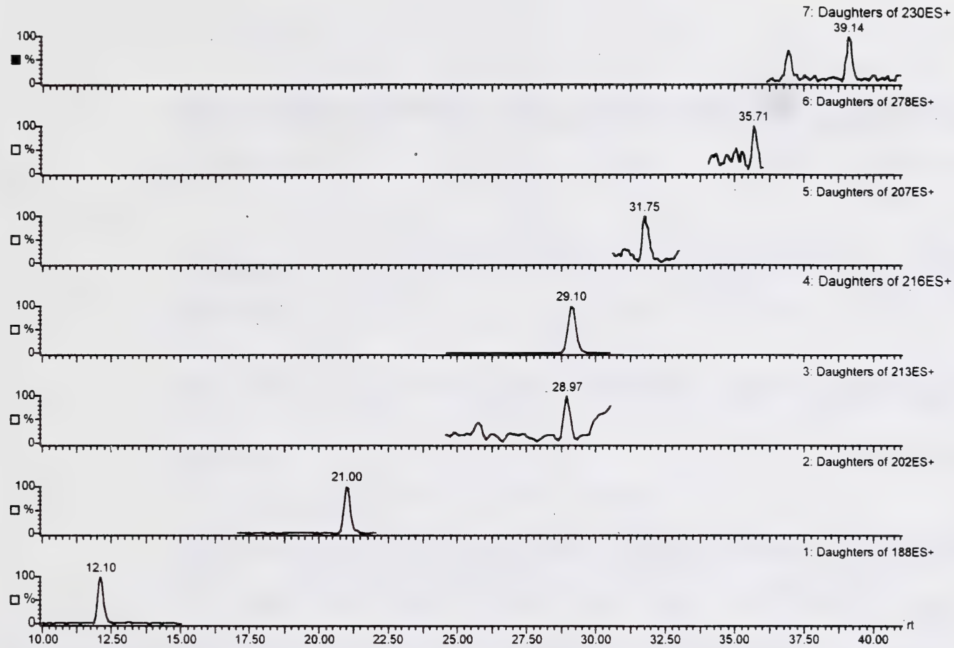
Library search from LC/MS/MS analysis (50pg on-column)



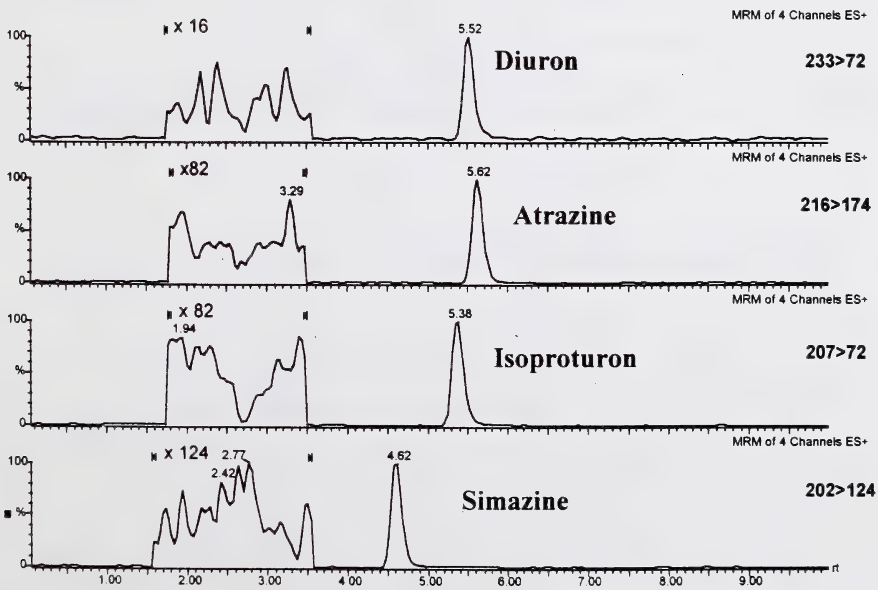
Library searching of daughter ion spectra from the analysis of surface water



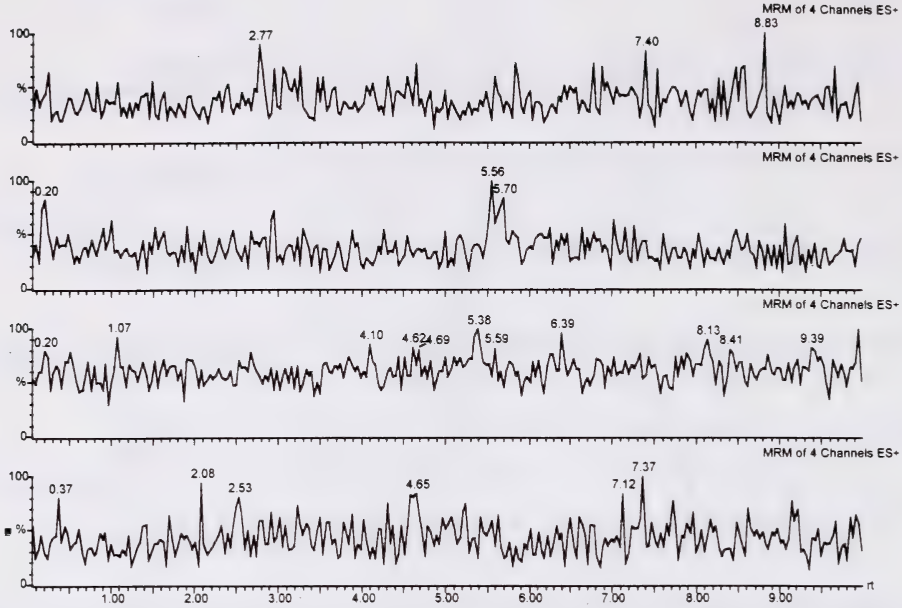
Daughter ion TICs from the targeted analysis of concentrated surface water 110



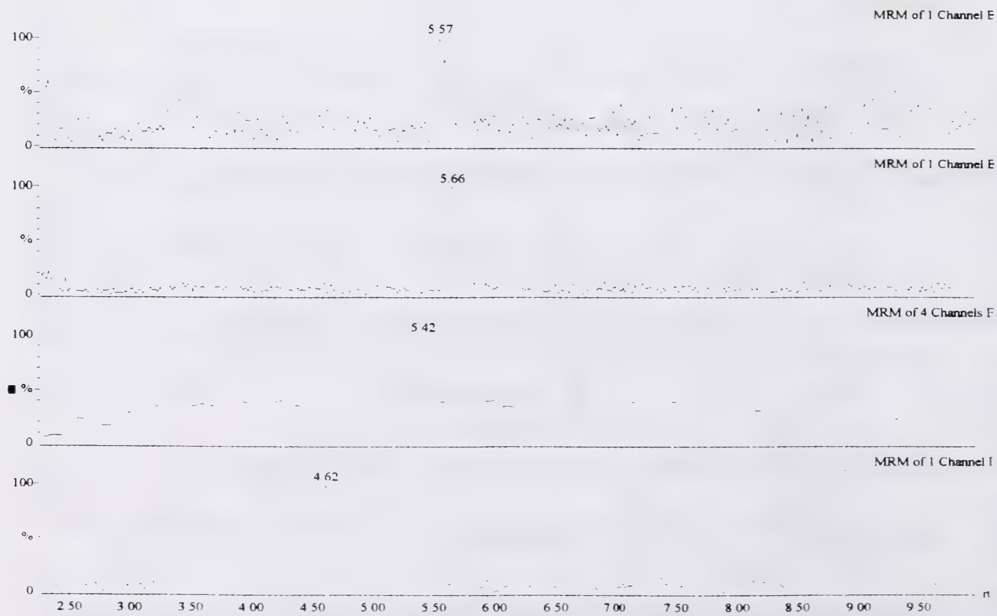
Daughter ion TICs from the targeted analysis of concentrated drinking water



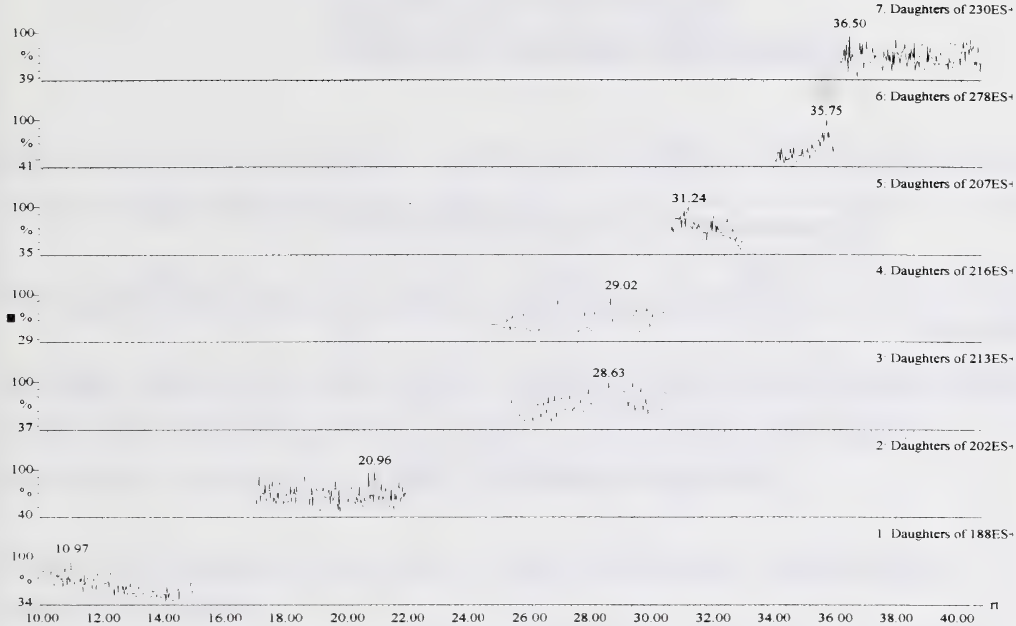
MRM analysis from a concentrated solution containing 10fg/µl of herbicides



MRM analysis of unconcentrated drinking water after activated carbon filtration



LC/MS/MS analysis of unconcentrated drinking water



Daughter ion TICs from the analysis of drinking water extracted through activated carbon filter

Quantification Results

Simazine Atrazine Isoproturon Diuron				
Drinking water	138	249	16	146
Surface water 050	30	72	18	33
Surface water 110	167	134	16	99

Values are in fg/ µl in water

Acknowledgements

Laboratorium antwerpse Waterwerken

Dr. F. Van Hoof

Mr. Ackermans Poe

Conclusions

Electrospray ionisation readily amenable to the analysis of a wide range of pesticide classes

Full scan TIC from LC/MS at 200pgs on-column

MS/MS facility allows highly specific characterisation of a pesticide enabling the creation of pesticide library for pesticide screening (LOD ~ 50pg on-column)

Fast Quantitative analysis of pesticides far below EEC directive levels

Experimental

olumn: Supelco LC-18-DB 25cm x 2.1mm

obile Phase: ACN/Water

gradient

TIME	% WATER	%ACN
0	80	20
30	65	35
60	90	10

ow: 200 μ/min. Eluent directed directly into source

ump: HP 1090 with autosampler

ample preparation: Zymark-SPE Supelco cartridge NVC18

S: Fisons VG Quattro II with MegaFlow Electrospray

Collision gas - argon $2x10^{-3}$ m bar

Full scan -150-300 in 2 sec. Continuum 8pts/Da

MRM - 0.5 secs (no span)

GLP - MYTHS AND FACTS

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With the enactment of US EPA FIFRA regulations 40 CFR part 160 (Oct. 16/89) changes were necessary in labs performing work to be submitted to EPA for pesticide registration. These changes involve all facets of the lab, which must be constantly monitored by a QA/AC unit. The quality of the final report in a GLP facility is supported by an increase in manpower and increase in costs.

GC/MS/MS ANALYSIS FOR PESTICIDES RESIDUES IN AGRICULTURAL PRODUCTS

BY

CARL FEIGEL
DANIELLE BELAND
VARIAN CHROMATOGRAPHY SYSTEMS

Title:
GC/MS/MS ANALYSIS FOR PESTICIDE RESIDUES IN AGRICULTURAL PRODUCTS.

The analysis of agricultural products for pesticide residues is of importance worldwide. This is because every country imports or exports food. Most countries do both. The issue that makes this important is that the pesticide limits set by the import country are almost always different than the limits of the country producing the product. Therefore, there is a need for reliable and accurate methodology for pesticide analysis. This presentation represents an initial study evaluating MS/MS as a superior technique to GC/MS analysis.

In this presentation, I will describe how ion trap MS/MS works in relation to another tandem MS/MS system--the triple quadrupole. I will also talk about the typical method development steps for optimizing the conditions. There will be some comparisons of MS/MS to both full scan EI analysis and to SIM analysis. Lastly, we will take a look at the quantitative data and method detection limits.

Objectives:
The objectives this project are several. First and most important is to determine if GC/MS/MS is well suited for this type of analysis. The reason that this analysis is difficult is because of the large amount of interference due to the chemical matrix. Sample clean-up usually needs to be extensive in order to accurately quantitate and identify pesticides at the low ppb level. This sample cleanup is time consuming, expensive, tedious, and can lead to errors and sample contamination. MS/MS, due to its extraordinary selectivity and sensitivity was evaluated as an alternative to this extensive sample clean-up. In order to determine the viability of MS/MS, detection limits and method development time need to be determined.

List of Pesticides
This list of 21 pesticides is hardly a complete list, but it represents many of the different types of pesticides that are used in different countries. There are some chlorinated hydrocarbons, nitroanalines, thiophosphates, and others. These pesticides were evaluated in 5 agricultural products: onion, broccoli, tomato, strawberry and oranges.

Ion Trap
For the benefit of those people who are more familiar with quadrupole mass spec, I will briefly describe how an ion trap works. This schematic shows the 3 basic parts to an ion trap: the upper and lower end caps and the center ring electrode. An RF frequency is placed on the ring electrode. This frequency will trap the ions in a confined space as soon as they are formed. These ions are formed in the same manner as with a quadrupole, but instead of repelling the ions away from the ionization zone, the ions are trapped where they are formed. These ions are than scanned out of the trap from low mass to high mass to collect the spectrum by raising the RF amplitude. In addition to the full scan process, there are ion isolation processes that are available. Frequencies can be placed on the upper and lower endcaps to select certain ions to be trapped and other ions to be selectively removed. Each particular m/z has a specific resonance frequency. If that specific frequency is applied, than that ion will gain energy and be ejected from the ion trap. In effect, the center ring electrode traps all of the ions, and the endcaps select some ions to be

ejected. This means that full scan, single or multiple ions, or clusters of ions can be selectively chosen for analysis.

Triple Quad
Now with this in mind, lets compare how a single ion trap can accomplish what a triple quadrupole does. The first stage of a triple quad is usually tuned to allow a single ion to pass through it. Traditionally this is called the parent ion. The parent ion exits the first quad and enters the second quad, which is usually referred to as the collision cell. This chamber is pressurized with Argon gas. The parent ion is accelerated and collides with the argon atoms. This impact causes collision induced dissociation (CID) and a spectrum of product ions are produced. This spectrum of ions exits the collision cell and enters the third stage. This last stage is for the mass analysis. The mass spectrum of the product ions is collected.

The single ion trap accomplishes all of this in the following manner. Ions are formed in the usual manner with the ion trap set to trap only the single ion of interest. Once this ion is isolated in the trap, frequencies are placed on the endcaps that cause the single ion to gain energy. When the ion gains energy, it accelerates its motion and eventually collides with a helium atom, present as the GC carrier gas. If the ion is accelerated enough, it will fragment just as in stage two of the triple quadrupole. The collision produces a spectrum of ions, which are immediately trapped by the frequencies on the ring electrode. The ion trap is then scanned in the usual manner from low mass to high mass to collect the product ion spectrum.

Method development
The step necessary for method development is to determine the proper energy that will fragment the parent ion. This is done by an automated software that allows the CID voltage to be incremented on a scan by scan basis. Simply set the scan speed to 10 scans per second such that scan one may be 20 volts and scan 2 would be 22 volts and so on. As we see in the first picture the amplitude is not high enough so the parent ion (231) remains intact. In the next scan the voltage is a little higher and the 231 ion is beginning to dissociate. In spectra 3 and 4 you can see a greater degree of dissociation.

Now we see a second example of the same process. As the voltage is increased more dissociation take place. From this injection, you can choose a specific CID voltage for each compound in the chromatogram.

Why is MS/MS necessary? (Dursban in orange extract)
Why is MS/MS a good choice for this analysis? What does it give you the GC/MS can't give you. Well, this is a mass chromatogram taken from the orange extract that I prepared. The ion trap is operated in the EI GC/MS mode. The small peak that you see corresponds to the retention time for the pesticide Dursban. The spectrum on the left is the spectrum of a Dursban standard. The one on the right is a background subtracted spectrum of the small peak. You first see that the signal-to-noise is very poor and the spectral match is not good enough to identify Dursban. The background matrix is so high that Dursban cannot be identified or quantitated with an certainty.

Dursban in orange with MS/MS

The next injection I made was the same sample analyzing it with MS/MS. You can see the tremendous improvement due primarily to the selectivity of MS/MS. The chemical matrix is removed and the signal-to-noise is almost 100 times better. The spectrum on the left is the MS/MS spectrum of a standard. The spectrum on the right is Dursban in the sample. If you analyzed this sample by GC/MS you would have erroneously stated a negative result when in fact Dursban is present in this sample. False negatives are possible when GC/MS is used for complex samples.

Ionization, isolation, and dissociation

Now I just want to reiterate the MS/MS process using this example to show how this selectivity process works. The ionization process produces all of the ions which are almost exclusively background matrix ions. We then isolate the parent ion (314) for the target compound (Dursban). Any background ions of m/z 314 are also isolated. All other ions are eliminated from the ion trap. The CID voltage that is optimized for the Dursban 314 ion is then applied to dissociate this ion. The resulting spectrum is free of interference. Any background ions at m/z 314 are either undissociated or they dissociate into masses other than m/z 258 or m/z 286 for Dursban. The signal-to-noise is improved due to the tremendous reduction in chemical noise.

Chlordane isomers

Another spectral advantage for MS/MS is the identification of isomers. The alpha and gamma chlordane spectra are identical in EI. Since the isomers have different structures, they are slightly different in internal energy. This means if you apply a constant CID voltage, the high energy isomer will dissociate to a greater degree as seen by the spectrum on the right. The lower energy isomer is just beginning to dissociate.

Instrument conditions

RTX5-amine column is a good choice for some of the more basic pesticides. The conditions for the Mass Spectrometer are not specific for this analysis. These are just autotune conditions.

MS/MS conditions

The parent ion was chosen due to its abundance. The base peak of an intense peak at a high m/z is always a good choice, but it isn't necessary or always convenient.

Calibration curves

The calibration curve is from 10 to 200 ppb which is a typical working range. In general, ion trap MS/MS is linear to a factor of 1000. In terms of material on column, I calibrated from 50 pg to 1000 pg. The ppb concentrations are based on the spike weight, sample weight and dilution factor.

Chromatogram of standard

This is a GC/MS/MS total ion chromatogram with 100 pg on column for each analyte. I am time programming the parent ion through the chromatogram and measuring the total product spectrum produced. The quantitation is based on an internal standard which is D-10 Phenanthrene.

Orange extract
The extracts are prepared by blending the sample, followed by a simple methylene chloride extraction. The extract is then filtered and concentrated to a known volume. I then spiked the extract so that the spike level was the equivalent of 20 ppb. This amount on column is 100 pg and the matrix is the soluble material from 5 mg of starting material. This is quite typical for pesticide analysis with the exception that there are sample cleanup steps that have been omitted. An MS/MS chromatogram is similar to a time-programmed SIM chromatogram. This makes sense since your time programming the parent ion. So how is this better than single Ion. Well the obvious improvement is that you get a spectrum to identify the compound. But there is a lot more.

Blow up of orange extract
If we look at the 4th peak which is about 375 scans, we see a very tall peak. This peak should only be about 1/3 as tall, which indicates that either there is some of this compound in the sample before the spike, or there is some positive interference. M/z 191 is the isolated ion. If we were doing single ion monitoring, we would see this large peak and quantitate this as a very high positive value. Only by doing MS/MS can this m/z 191 interference be separated form the m/z 191 of Chlorneb.

MS/MS of unspiked orange extract
If we look at the unspiked extract you can see the major interference that is present. The m/z 191 ion fragments into a different pattern than the pesticide Chlorneb. Notice that the interference spectrum does not contain the m/z 163 ion which is specific to the MS/MS spectrum of Chlorneb. The selectivity of MS/MS allows this--full scan EI or SIM would lead to a false positive result.

GC/MS/MS mass chromatogram of m/z 163 (Chlorneb)
With MS/MS we dissociate the 191 ion to its product spectrum. There are 2 ions present in the ion trap, both with m/z values of 191. One of these ions is from Chlorneb and the other is a background ion from the sample. When m/z 191 dissociates, it dissociates into two different spectra specific to the structure of the ions. M/z 163 is present in the Chlorneb dissociation pattern but not in the background compound pattern (see previous slide). The total ion chromatogram represents all of the ions from the dissociation of m/z 191. The mass chromatogram of m/z 163 represents the ions due to Chlorneb. This peak area can be measured and quantitated to accurately determine the Chlorneb concentration.

Quantitative results
The spike level is 20 ppb, and you can see that these are very acceptable results. This is much better than could be achieved in full scan or single ion due to the interferences. Notice the accuracy of the Chlorneb in orange extract with the coeluting m/z191 compound.

Method detection limits
The MDL's are calculated by making a series of replicate injections and determining the standard deviation from the results. The standard deviation is multiplied by the Student T factor for the 99% confidence level. This is the value reported as the MDL. For the standards, 1-10 ppb is detectable depending on the compound. In 4 of the 5 extracts the MDL for the extracts is

comparable to the standards MDL. The broccoli extract is the notable exception. The data suggest that some additional optimization might be necessary for this extract. Notice the MDL for Chlorneb in the orange extract. Even with the large interference coeluting with Chlorneb, the MDL is not statistically different form the standard or the other extracts. This is due to the selectivity of MS/MS.

Conclusion

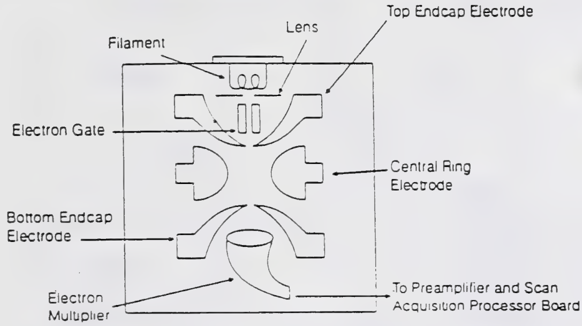
GC/MS/MS analyses are accurate and reproducible in these complex matrices. Additional optimizations may be necessary for certain compounds in certain matrices. Samples can be analyzed without extensive and costly cleanup steps. MDL's of 10 ppb or less is obtainable for many pesticides.

OBJECTIVES

1. **EVALUATE GC/MS/MS FOR PESTICIDE RESIDUE ANALYSIS.**
2. **DETERMINE TYPICAL METHOD DEVELOPMENT TIME.**
3. **CALCULATE METHOD DETECTION LEVELS.**
4. **DETERMINE REPRODUCIBILITY AND ACCURACY AT LOW LEVELS IN EXTRACTS WITH NO SAMPLE CLEANUP.**

LIST OF PESTICIDES

DICHLOBENIL	ETRIDIAZOLE	CHLORNEB
METHYLNAPHTHALENE	LINDANE	CLOMAZONE
CHLOROTHALONIL	TERBUFOS	MALATHION
CHLOROPROPYLATE	DURSBAN	OXADIAZON
α-CHLORDANE	χ-CHLORDANE	FAMPHUR
METHOXYCHLOR	DDT	ETHION
DICHLOFENTHION	DIPHENAMID	TRIFURALIN



ION TRAP STEP 1

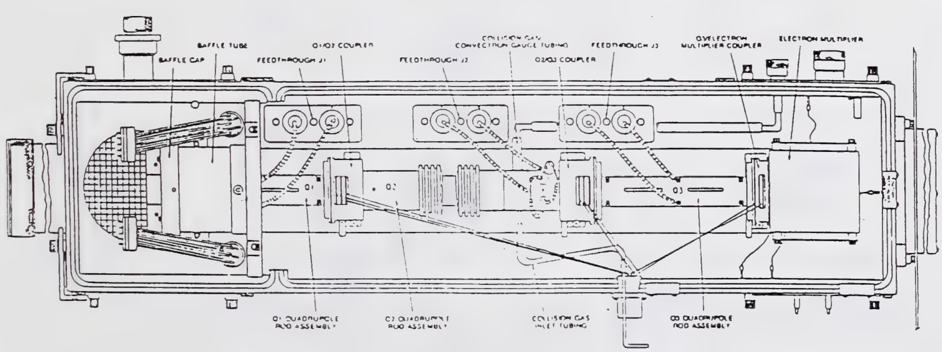
Ions are formed in the center of the trap. Waveforms placed on the end caps eject all unwanted ions. The target ion is stored in the ion trap by the RF trapping field.

ION TRAP STEP 2

A second waveform excites the isolated trapped ion. This causes energetic collisions with the helium carrier gas. The target ion is fragmented and the resulting product ions are trapped by the RF trapping field

ION TRAP STEP 3

The RF field is scanned in the normal manner. The unreacted target ions and the product ions are recorded by the electron multiplier. The entire 3 step process occurs in 0.1-0.2 seconds.



QUADRUPOLE STAGE 1

Ions are formed in the ion source and focused into the quadrupole which is tuned to allow a single target ion to pass through to the second stage.

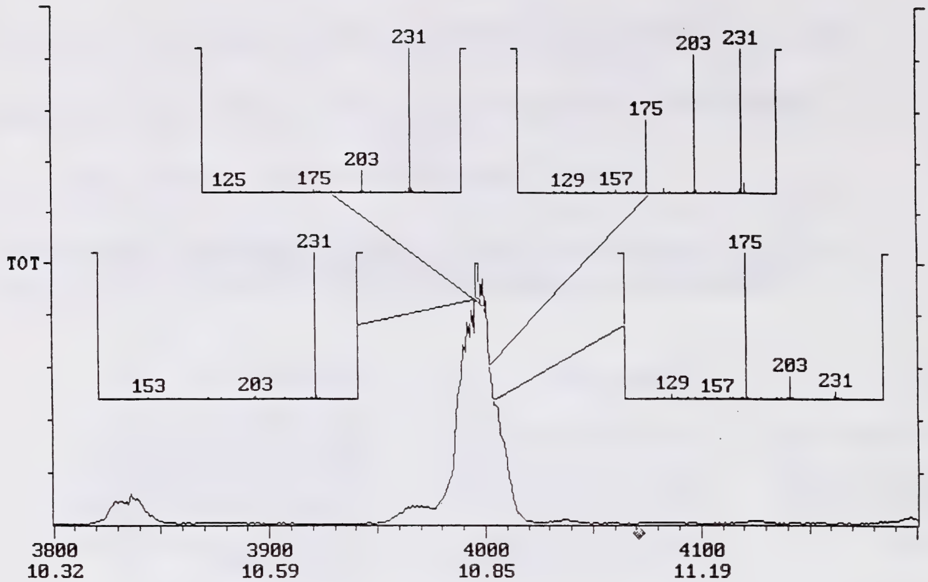
QUADRUPOLE STAGE 2

The single m/z ions exiting stage 1 travel to stage 2 which is slightly pressurized with Argon. Energetic collisions take place and the target ion is fragmented. This is called collision induced dissociation

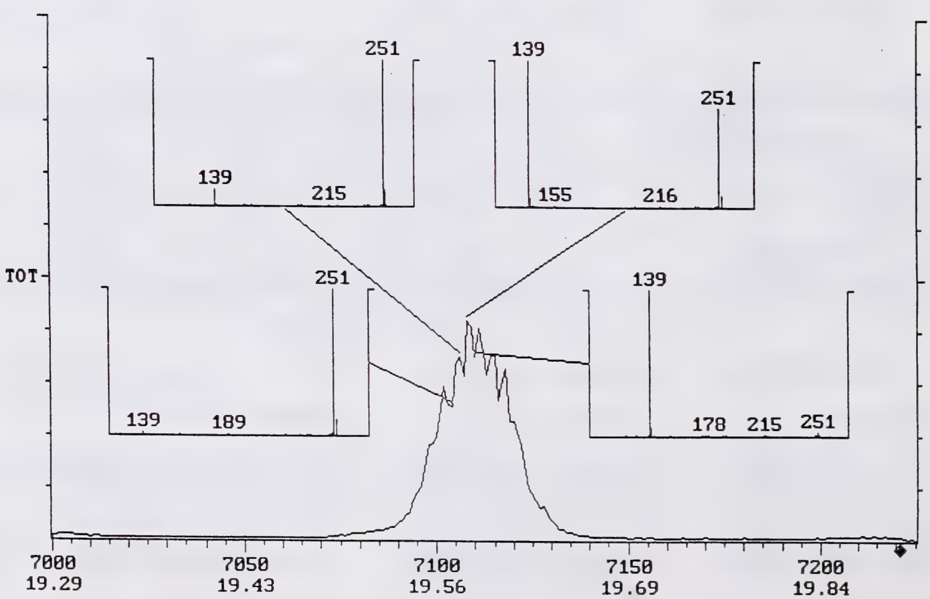
QUADRUPOLE STAGE 3

The unreacted target ion and all CID product ions enter the third stage where the mass filter separates the ions. The tandem mass spectrum is recorded by the electron multiplier.

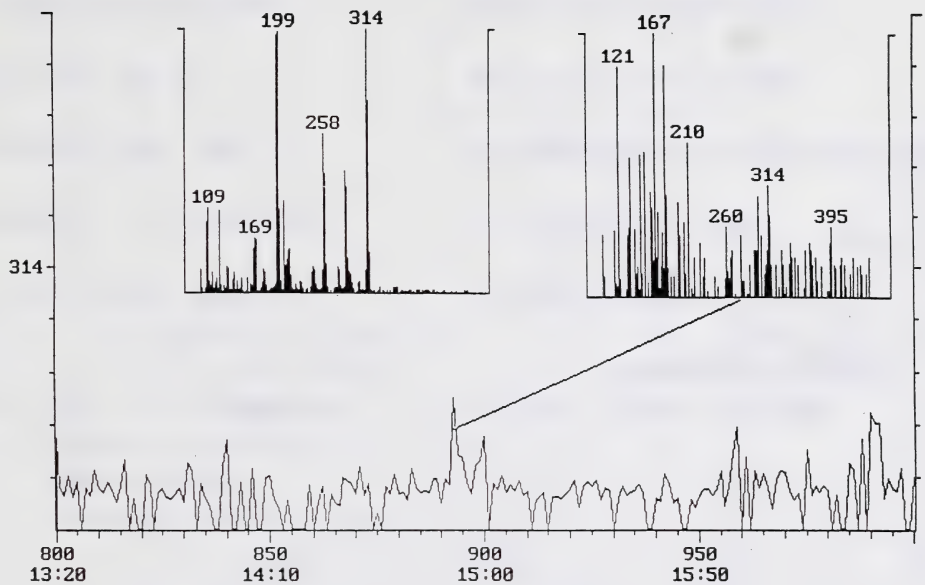
TERBUFOS (PARENT ION M/Z 231)



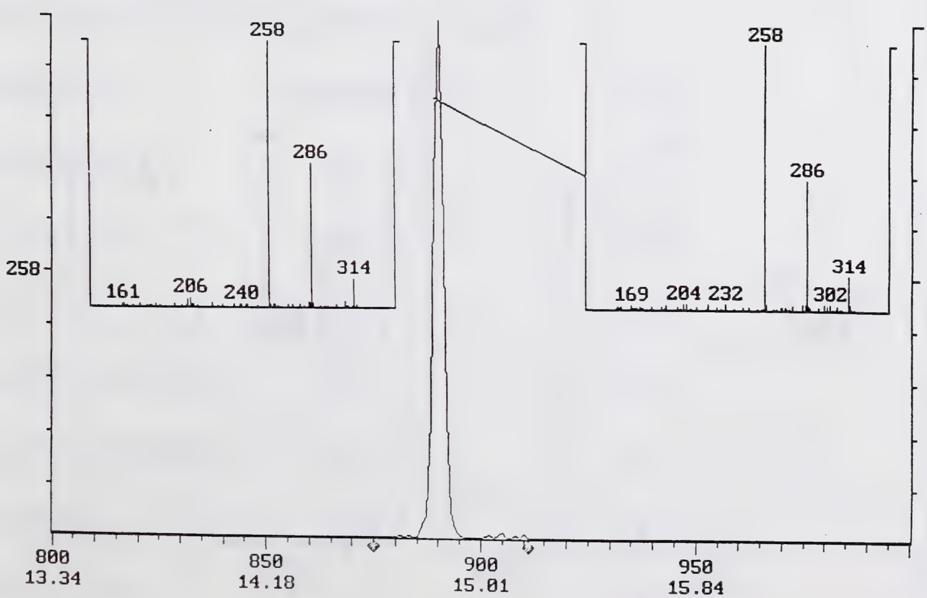
CHLORPROPYLATE (PARENT ION M/Z 251)

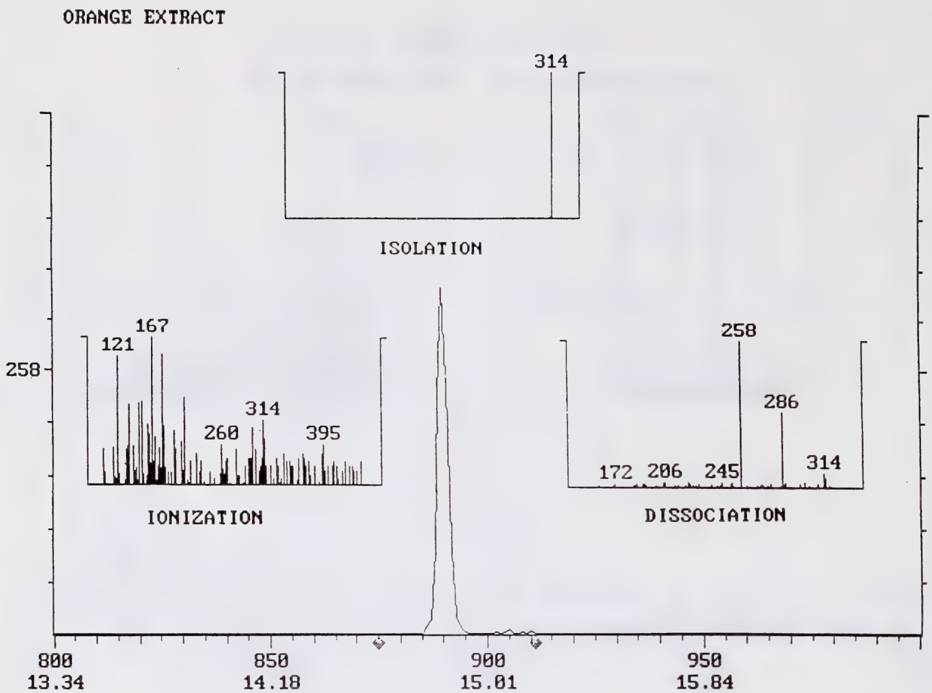


ORANGE EXTRACT EI-MS MASS RANGE 100-450



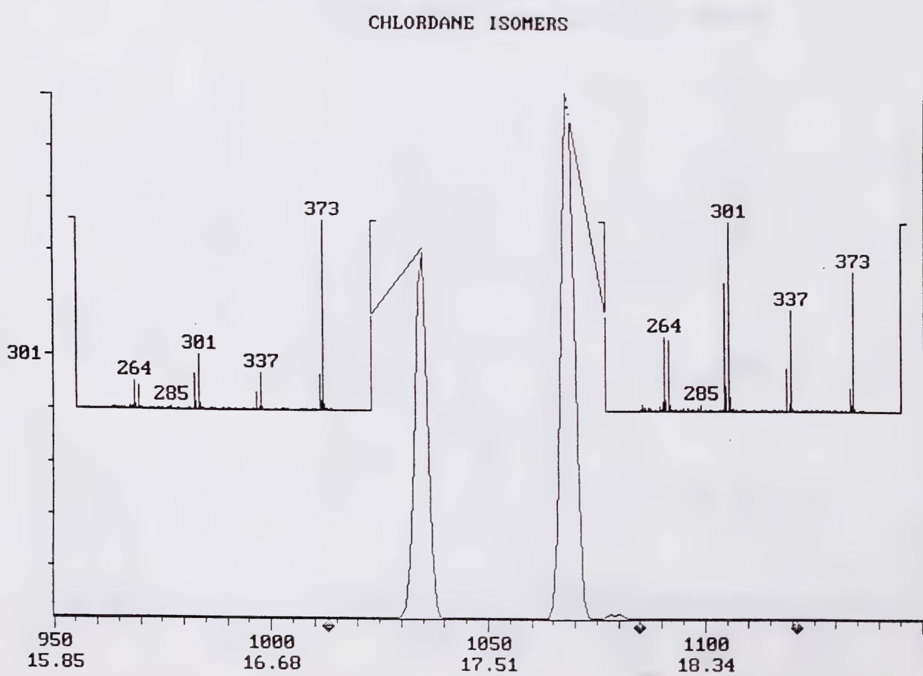
DURSBAN IN ORANGE EXTRACT WITH GC/MS/MS





ORANGE EXTRACT
314
ISOLATION
121
167
260
314
395
IONIZATION
258
286
172
206
245
314
DISSOCIATION
258
800
13.34
850
14.18
900
15.01
950
15.84

FIGURE 1



CHLORDANE ISOMERS
373
264
301
285
337
301
264
285
337
373
301
950
15.85
1000
16.68
1050
17.51
1100
18.34

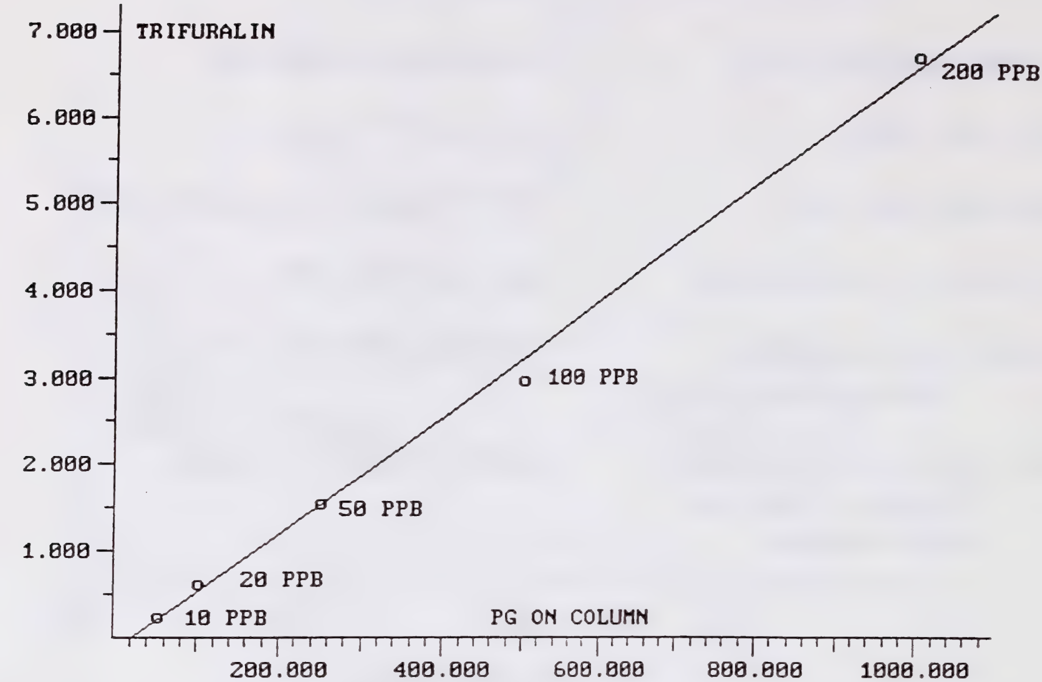
INSTRUMENT CONDITIONS

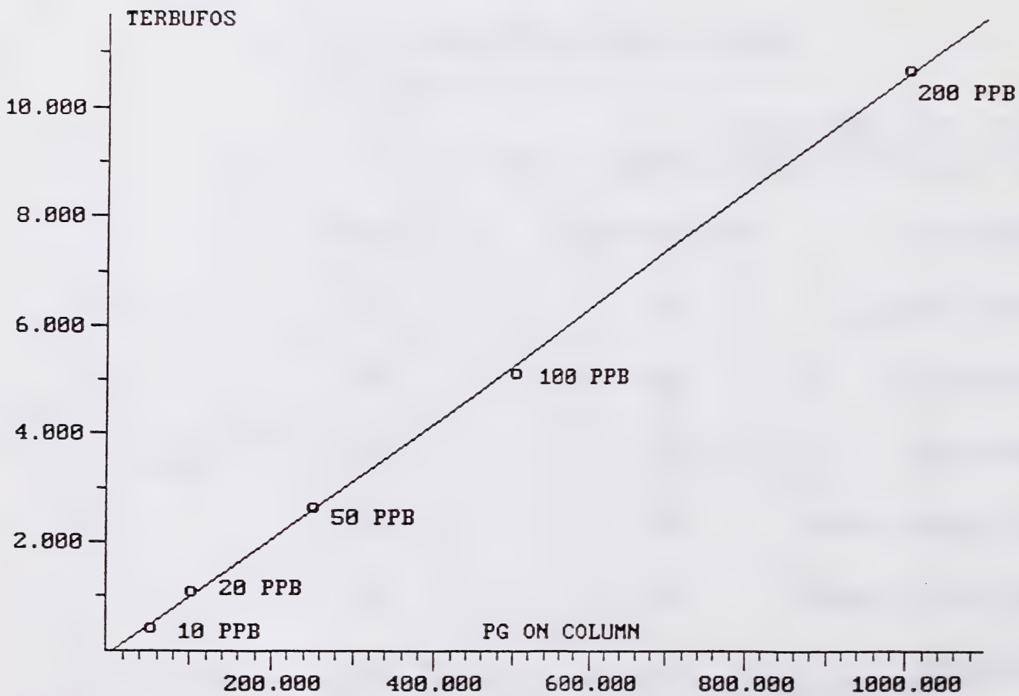
GAS CHROMATOGRAPH	MASS SPECTROMETER
INJECTOR: 280° C	FILAMENT: 90 μAMPS
TRANSFER LINE: 280° C	MULTIPLIER: AUTOTUNE +100 V
INJECTION: 2 μL SPLIT 20:1	AGC TARGET: 5000
COLUMN: RTX-5 AMINE 30m x 0.25mm x 0.5μm	MANIFOLD TEMP: 220° C
OVEN: 150°C for 0.1 min. then 5°C/min. to 300°C and hold for 10 min.	MODULATION: 4 VOLTS

MS/MS CONDITIONS

MODE: NON-RESONANT
EXCITATION STORAGE LEVEL: 75 AMU

COMPOUND	PARENT ION	VOLTS
ETRIDIAZOLE	211	57
TRIFURALIN	306	45
TERBUFOS	231	54
CHLORTHALONIL	266	71
CHLORFENTHION	279	54
DURSBAN	314	42
OXADIAZON	258	42


TRIFURALIN
7.000
6.000
5.000
4.000
3.000
2.000
1.000
200 PPB
100 PPB
50 PPB
20 PPB
10 PPB
PG ON COLUMN
200.000
400.000
600.000
800.000
1000.000


TERBUFOS
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8.000
6.000
4.000
2.000
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20 PPB
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PG ON COLUMN
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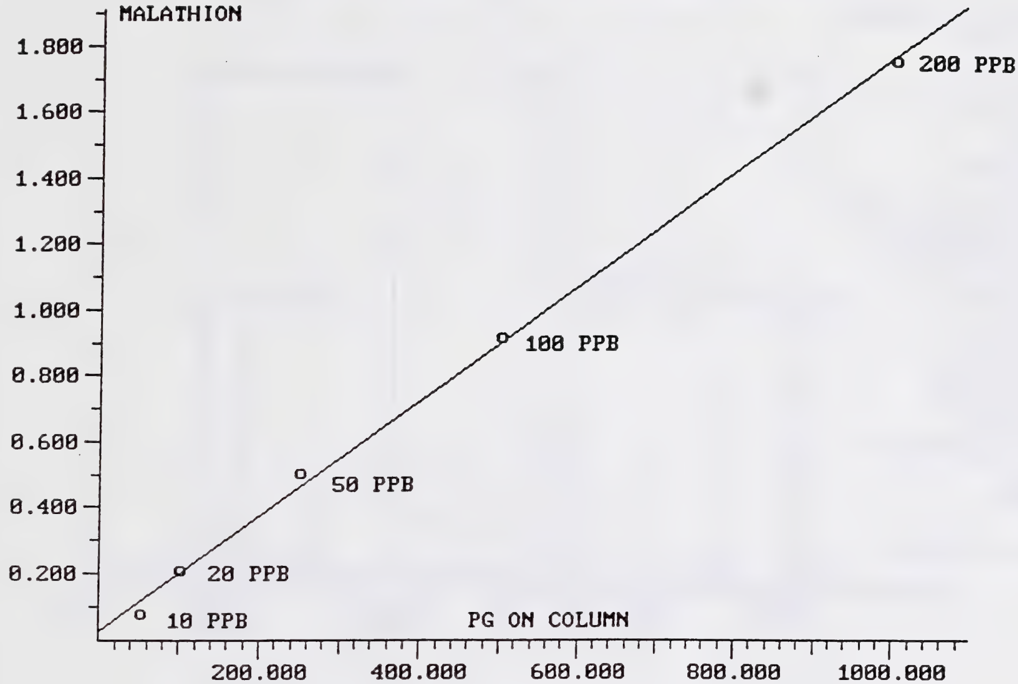
400.000
600.000
800.000
1000.000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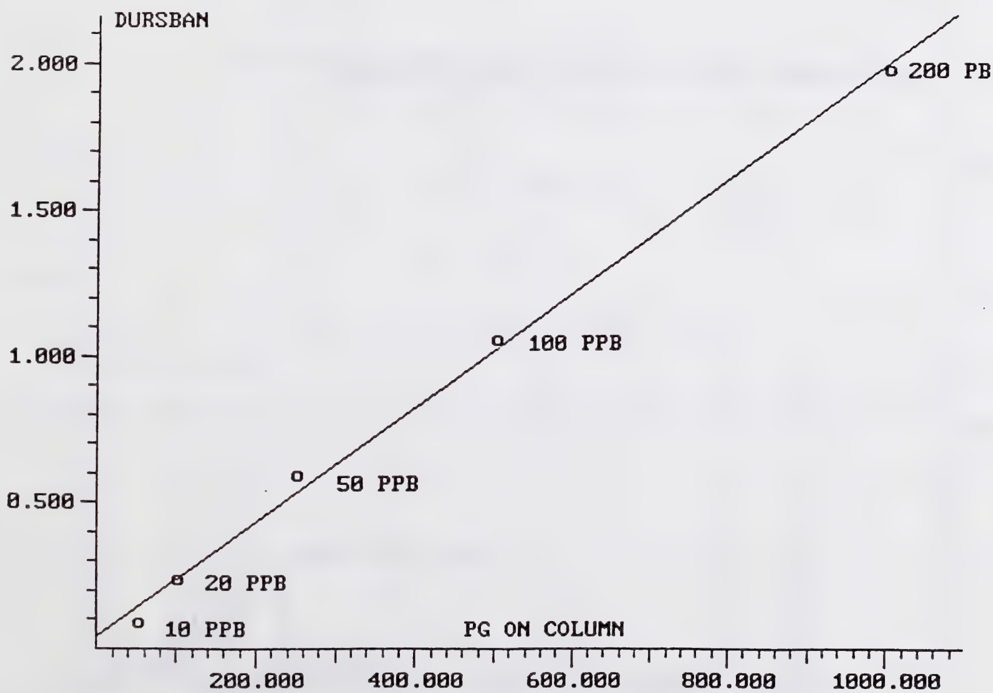
1.600
1.400
1.200
1.000
0.800
0.600
0.400
0.200
200 PPB
100 PPB
50 PPB
20 PPB
10 PPB
PG ON COLUMN
200.000
400.000
600.000
800.000
1000.000


DURSBAN
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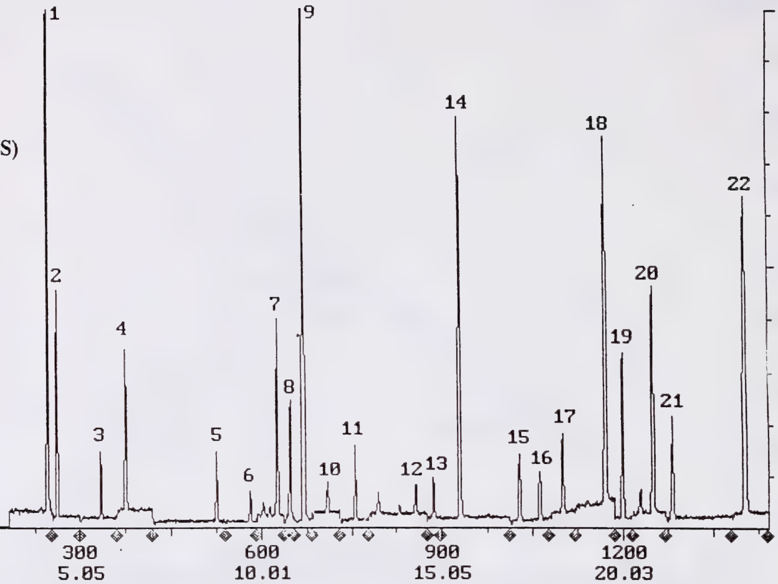
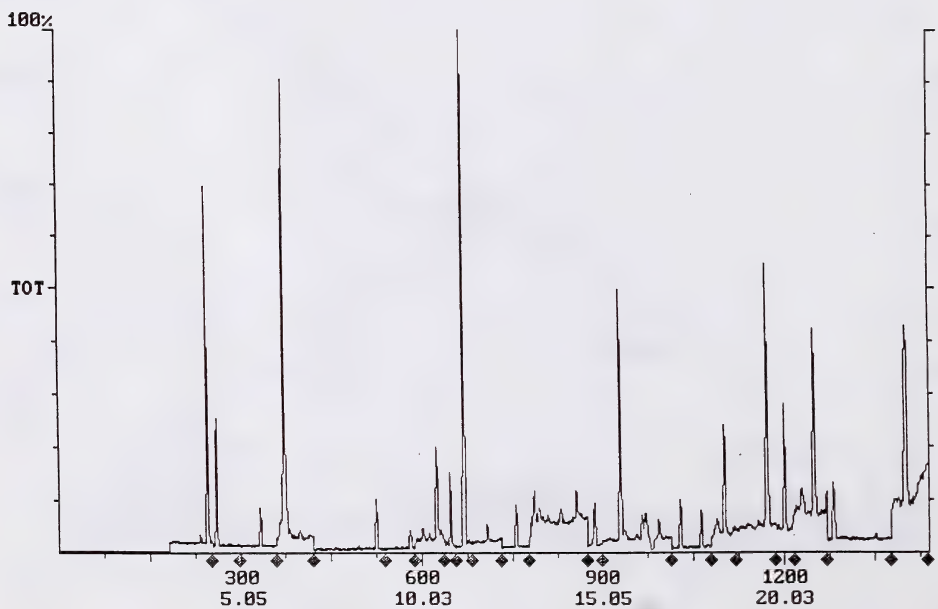
1.500
1.000
0.500
200 PB
100 PPB
50 PPB
20 PPB
10 PPB
PG ON COLUMN
200.000
400.000
600.000
800.000
1000.000

PESTICIDE STANDARD BY GC/MS/MS (100 PG ON COLUMN)

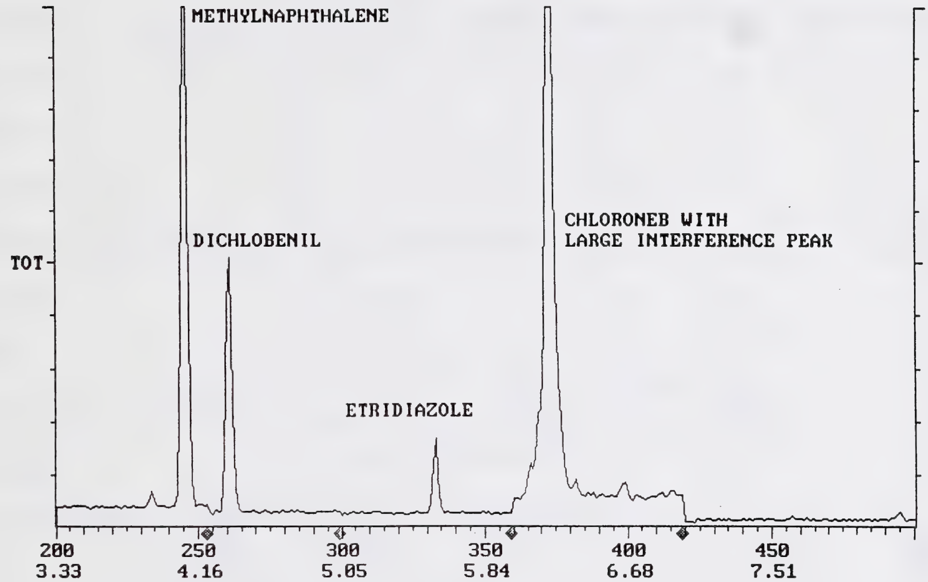
1. METHYLNAPHTHALENE
2. DICHLOBENIL
3. ETRIDIAZOLE
4. CHLORNEB
5. TRIFURALIN
6. LINDANE
7. CLOMAZONE
8. TERBUFOS
9. PHENANTHRENE D-10 (IS)
10. CHLOROTHALONIL
11. DICHLOFENTHION
12. MALATHION
13. DURSBAN
14. DIPHENAMID
15. α-CHLORDANE
16. χ-CHLORDANE
17. ODADIAZON
18. CHLOROPROPYLATE
19. ETHION
20. FAMPHUR
21. DDT
22. METHOXYCHLOR



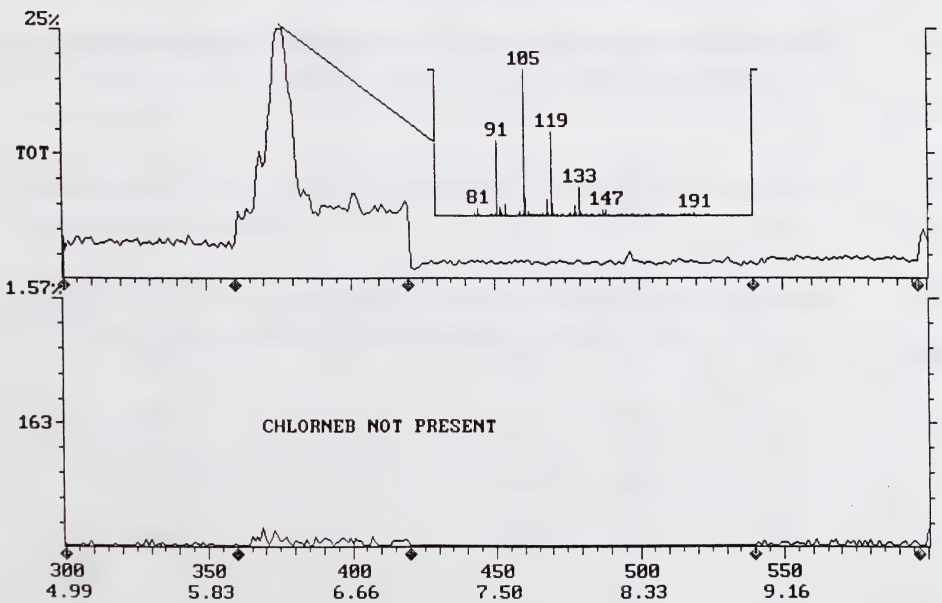
ORANGE EXTRACT SPIKED WITH 20 PPB (100 PG ON COLUMN)



ORANGE EXTRACT SPIKED WITH 20 PPB (100 PG ON COLUMN)



GC/MS/MS OF UNSPIKED ORANGE EXTRACT



ORANGE EXTRACT SPIKED WITH 20 PPB (100 PG ON COLUMN)

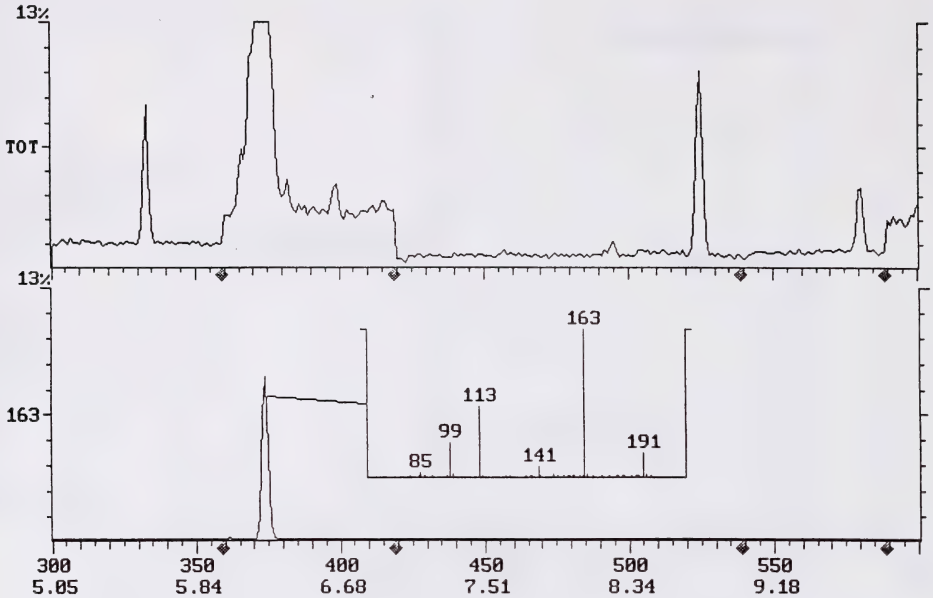


TABLE 1

RECOVERY OF 20 PPB SPIKED EXTRACTS (N = 8)

COMPOUND	BROCCOLI	ONION	TOMATO	ORANGE	STRAWBERRY
ETRIDIAZOLE	12	16	22	22	21
CHLORNEB	20	19	18	26	22
TRIFURALIN	22	18	16	27	19
CLOMAZONE	18	15	15	21	23
MALATHION	14	15	15	23	21
DURSBAN	22	19	16	22	25
FAMPHUR	25	26	20	27	21
OXADIAZON	22	23	18	25	23
α-CHLORDANE	19	20	16	26	23

TABLE 2

METHOD DETECTION LIMITS IN PPB (N=8)

COMPOUND	STANDARD	BROCCOLI	ONION	TOMATO	ORANGE	STRAWBERRY
ETRIDIAZOLE	2	12	3	5	6	3
CHLORNEB	1	2	2	2	3	2
TRIFURALIN	6	4	2	2	4	3
CLOMAZONE	2	7	1	2	5	1
MALATHION	3	8	2	1	3	3
DURSBAN	2	2	2	2	7	2
FAMPHUR	1	16	2	3	5	2
OXADIAZON	2	7	2	2	2	2
α-CHLORDANE	3	9	2	3	4	2

CONCLUSION

1. **METHOD DEVELOPMENT IS SIMPLE AND CAN BE DONE IN A TIMELY MANNER EVEN FOR A LARGE NUMBER OF ANALYTES.**

2. **PESTICIDES CAN BE ANALYSED ACCURATELY WITHOUT SAMPLE CLEANUP.**

3. **METHOD DETECTION LIMITS OF 1-10 PPB (5-50 PG) ARE TYPICAL FOR PESTICIDES IN REAL SAMPLES.**

SUMMARY OF PRESENTATION TO

WESTERN TRACE ORGANIC/PESTICIDE RESIDUE WORKSHOP

May 8 and 9, 1995
Edmonton, Alberta

EXPERT WITNESSES: EVIDENCE GIVING IN COURT PROCEDURE

I hope that I will be able to offer some practical advice on what to expect when you appear as an expert witness and how to handle cross-examination. This will not be a lecture on the law. It will be more useful to discuss what it is that makes for a good expert witness and to hopefully dispel some of the myths associated with court testimony. I have heard such various comments as:

"you will be ripped apart on cross-examination"

"you should only answer with a yes or no answer and say as little as possible"

"you have an image to uphold"

"the witness is on trial"

"the opposing counsel is the enemy"

Fortunately, such views are simply wrong. They are, I suspect, the product of what we see on television. Court is not a strange place. The same things that make any professional experience a nightmare will make Court horrible, specifically:

1. lack of preparation

2. a misunderstanding of your role.

I hope to address these two issues in a very practical way.

Typically, you do not choose to become an expert witness. The opportunity arises because of the work that you do. It is my hope that you would view the experience as an opportunity for your work to be recognized rather than an occasion that fills you with dread.

Susan McRory
Coordinator of Environmental Prosecutions
for the Province of Alberta
Special Prosecutions Branch

DETERMINATION OF CLOPYRALID, PICLORAM AND SILVEX AT LOW CONCENTRATION IN SOILS BY CALCIUM HYDROXIDE-WATER EXTRACTION AND GAS CHROMATOGRAPHY MEASUREMENT

Liang K. Tan, David Humphries, Paul Y.P. Yeung, L. Zack Florence

Alberta Environmental Centre
Vegreville, AB T9C 1T4

Clopyralid, picloram and silvex have been determined at 10.0 ng/g level in soil by extraction with calcium hydroxide and water, followed by partition of the herbicides from the aqueous phase to dichloromethane phase and then derivatization of the residues with diazomethane. The methyl ester derivatives were measured by GC/ECD. Alberta soils of various types were fortified with known quantities of these herbicides and were analyzed. The use of divalent CA^{2+} ion to precipitate humic matter and leaving the herbicides in the basic aqueous phase lead to the absence of emulsion in the subsequent liquid-liquid partition process. The importance of pH for a successful recovery in this partition process is discussed. Precision of analysis at 10.0 ng/g is 1 to 13%. Herbicide recoveries from fortified soils are compared and discussed in relation to the soil components. Clopyralid recovery is 95.2±6.7%) and is independent of organic matter (0.4 to 10.1%), clay (3.6 to 44.2%), sand (16.5 to 94.1%) or iron (3.9 to 22.4 mg/g) contents in the soils. Picloram and silvex recoveries (58.0 to 97.8%) are dependent on soil types. Detection limits (2.5 to 50.0 ng/g) are dependent on the herbicide and soil type. Lower recoveries of herbicides as a result of drying fortified soil slurries are also discussed.

DETERMINATION OF HERBICIDE RESIDUES IN LIPID RICH-TISSUE USING MS/MS

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INTRODUCTION

An investigation of the distribution of the levels of contaminants within a population of amphipods exposed to pesticide residues in wetland environments was conducted in our laboratory. For this investigation, an analytical method was required for (a) the confirmation of the presence of contaminants in the individual amphipods, (b) the assessment of the distribution of contaminants within the lipid reserves of the amphipods and (c) complementary broad spectrum analyses[1,2] to conventional techniques. To meet these objectives, the utility of tandem mass spectrometry (MS/MS) was evaluated for specific application to the limited amounts (less than 1 mg weight) of lipid- rich tissue, exposed to triallate and diclofop-methyl in prarie wetlands.

EXPERIMENTAL

MATERIALS

Authentic standards were obtained from Monsanto and Hoechst Canada for triallate and diclofop-methyl, respectively. These standards were used to obtain library spectra for confirmation of the identity of the herbicides and their transformation products in amphipod tissue.

PROCEDURE

The design, construction and operation of the field microcosms have been reported earlier[2]. The microcosms were composed of a wooden frame and polyethylene side walls enclosing a volume of 1 m^3. Five of the microcosms were spiked with triallate (Avadex®-BW) and five with diclofop-methyl (Hoe Grass®) in a log series (0.01 to 100 mg/L of active ingredient). One of the microcosms served as a control. Adult amphipods (*Gammararus lacustris* Sars) were collected form the microcosms and lipid-rich tissue was obtained by the procedure described earlier, Arts et al. (in review). Preliminary MS experiments were conducted using a Fisons AutospecQ mass spectrometer with EBEQ geometry, equipped with a 4000-60 VAX data system, Digital Equipment Co., and Opus V3.1X software. Instrumental parameters are described elsewere[4]. In brief, samples were placed in shallow cups of the direct insertion probe for 30 minutes at room temperature (approximately 23°C), prior to introduction to the ion-source. This was necessary to reduce the moisture content of the samples and avoid tripping the vacuum protection system (set at

5 x 10-5 torr) of the mass spectrometer. The direct insertion probe was water-cooled, and heating was limited to the radiant heat from the ion-source with no additional heat supplied by the probe heaters. The ion-source was operated under electron impact conditions at 70 electron volts, 250°C, trap current 250 mA, and the mass spectrometer was operated at 1300 resolution, with a scan speed 1 sec/decade, and mass range 50 - 600 daltons.

For the MS/MS experiments, the precursor ions were selected manually, and the ion-beam transmission was reduced to 50% using Xenon as the collision gas while transmitting m/z 331 from perfluorokerosene . The collision cell was located in the fourth field free region. Experiments were performed for low energy collisions in which the collision cell was held at 12 eV (laboratory frame of reference). Product-ions were detected by scanning the quadrupole in the mass range 30-350 daltons at unit resolution[5].

RESULTS AND DISCUSSION

The electron impact mass spectra of the authentic standards of the herbicides were first examined to select diagnostic ions suitable for evaluating the presence of the target analytes in the full scan mass spectra of amphipod tissue. Product-ion spectra were used to (a) verify that the selected extracted-ions were diagnostic for the respective analytes, and (b) enhance the sensitivity of the method for identification of the herbicides , relative to the MS full scan procedure.

Prelimanary results of the product-ion scans of representitive tissue samples indicated that triallate could still be detected after 30 days in the10 mg/L treatment but was not detected in the control microcosm. Preliminary data obtained for diclofop methyl also confirmed the presence of the herbicide in the lipid-rich tissue of the amphipods.

CONCLUSION

The detection limit of the tandem mass spectrometry technique was suitable for the confirmation of herbicides in the individual amphipods and served as a complementary tool to conventional analyses of herbicide residues in the water and sediment compartments.

ACKNOWLEDGEMENTS

Technical assistance was provided by Mary Ferguson.

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ANALYSIS OF RESIN- AND FATTY ACIDS

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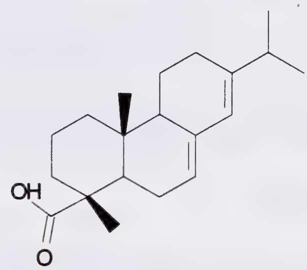
INTRODUCTION

Resin- and fatty acids (RFAs) are a group of approximately 22 compounds which are extracted from wood during debarking and pulping, and have been detected in the effluent of many pulp and paper mills. In some cases resin acids (RAs) are the major source of fish toxicity in mill effluent. Therefore, the analysis of RFAs has been an important element in the Canadian pulp mill water quality monitoring program.

The diterpenoid structure of RAs is illustrated below for abietic acid. The fatty acids are aliphatic or unsaturated straight-chain molecules containing from 12 to 24 carbon atoms.

Resin acids may be analyzed in water by extracting the acidified (pH 2) sample with dichloromethane[1], or under basic conditions (pH 9) using diethyl ether[2] or methyl t-butyl ether (MTBE)[3].



abietic acid

METHOD SUMMARY

Water samples (100 mL) were extracted 3 times with 50-ml portions of organic solvent. Extracts were filtered through anhydrous sodium sulfate, concentrated to ~ 1 mL, and treated with diazomethane solution. The derivatized extract was analyzed by gas chromatography using a DB-5 capillary column with flame ionization detection.

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[1] B.C. Ministry of Environment. "RFAs in water"

[2] Environ. Canada Labs. Pacific & Yukon Region. "Resin Acids". V2.5, April 1994

[3] Lee H-B, Peart TE, Carron JM. *J. Chromatog. 498,* 367, (1990).

Table 1: **Spiking levels for method detection limits, and analyte codes**

Compound	Code	Spike Level (ug/L)
LAURIC	C12	0.2
MYRISTIC	C14	0.3
PALMITIC	C16	0.3
5-a-ANDROSTANE (internal std.)	AN	0.7
LINOLEIC	C18,2=	0.2
LINOLENIC	C18,3=	0.2
STEARIC	C18	0.3
NONADECANOIC	C19	0.2
SANDARACOPIMARIC	SAN	0.2
EICOSANOIC	C20	0.2
ISOPIMARIC	i-PIM	0.2
LEVOPIMARIC	L-PIM	0.4
DEHYDROABIETIC	DAB	0.2
ABIETIC	AB	0.3
NEOABIETIC	NAB	0.3
DOCOSANOIC	C22	0.3
14-CHLORODEHYDROABIETIC	14Cl	0.5
12-CHLORODEHYDROABIETIC	12Cl	0.5
LIGNOCERIC	C24	0.2
12,14-DICHLORODEHYDROABIETIC	DiCl	0.3

RESULTS

"Acidic extraction" was initially examined. Distilled water samples were acidified to pH 2 and extracted with dichloromethane. Recoveries of FAs from distilled water were generally acceptable, but recoveries of several RAs were unacceptably low in our hands (Figure 1). No neoabietic acid was recovered, and the recovery of levopimaric acid was ~ 30 % . "Basic extraction" was then examined in which distilled water samples were basified to pH 9 and extracted with diethyl ether. This method also showed unacceptably low recoveries for some analytes (Figure 2). "Neutral extraction" of distilled water was then examined using dichloromethane. Recoveries of all analytes except levopimaric acid (~ 50 %) were acceptable (Figure 3). In a direct comparison of pH 7 and pH 9 distilled water extractions, both using dichloromethane, recoveries of RFAs were slightly or significantly higher at pH 7 (Figure 4). When neutralized (pH 7) marine water was extracted (using dichloromethane), low recoveries were observed for several of the FAs, whereas all of the RAs were recovered satisfactorily (Figure 5). Pulp mill (final discharge) effluent was then examined. Effluent samples were buffered to pH 7; the concentration of phosphate buffer was 0.2 M in the samples. Recoveries of spiked RFAs from buffered mill effluent were acceptable, similar to those from unbuffered (pH7) distilled water (Figure 3). This prompted the determination of method detection limits (MDLs) involving the analysis of 8 buffered effluent samples spiked at "low" levels (Table 1).

Preliminary results suggested that the MDLs for RFAs in this effluent were 0.2 to 0.5 ug/L. These were acceptable considering the relatively small sample size of 100 mL.

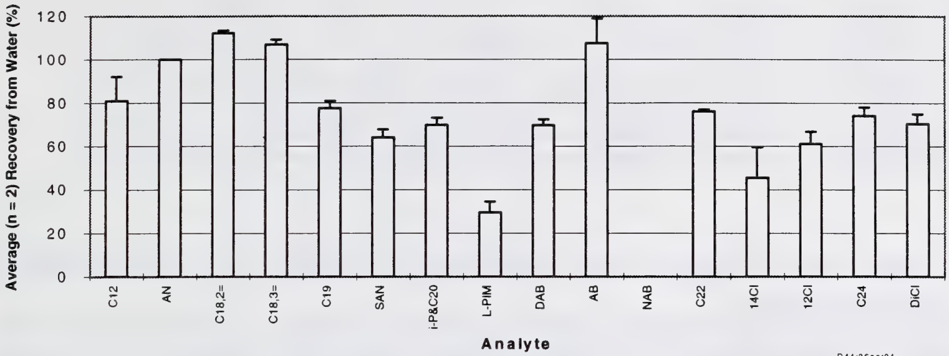

Figure 1. Recoveries of spiked RFAs from distilled water at pH 2 using dichloromethane.

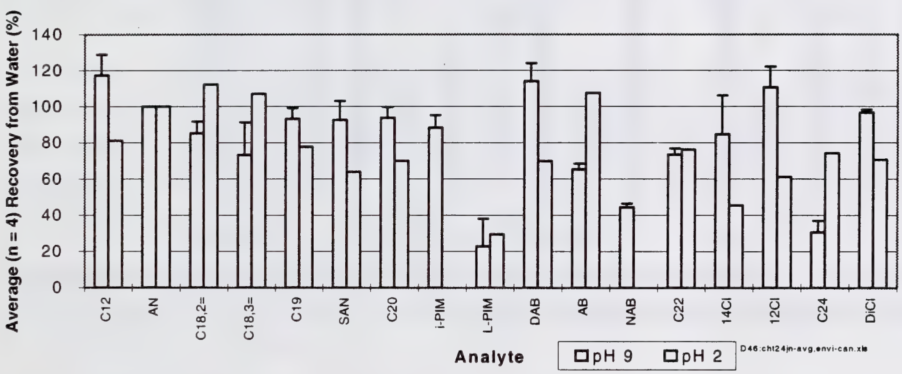

Figure 2. Recoveries of spiked RFAs from distilled water at pH 9 using diethyl ether. pH 2 results (Figure 1) also plotted.

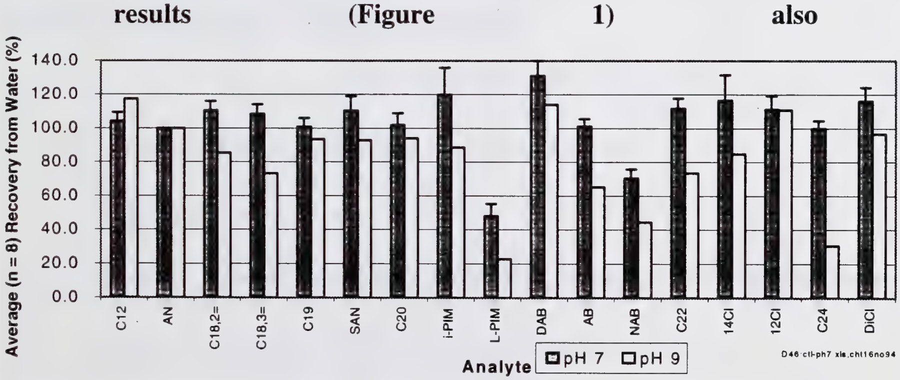

Figure 3. Recoveries of spiked RFAs from distilled water at pH 7 using dichloromethane. pH 9 results (Figure 2; using ether) also plotted.

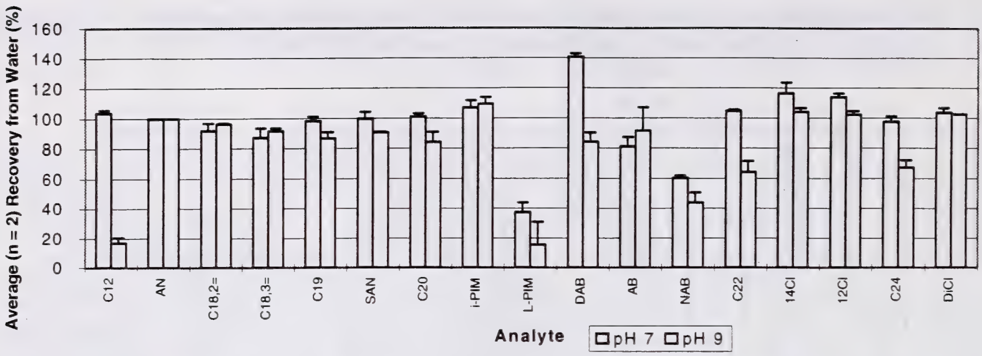

Figure 4. Recoveries of spiked RFAs from distilled water at pH 7 and 9 using dichloromethane.

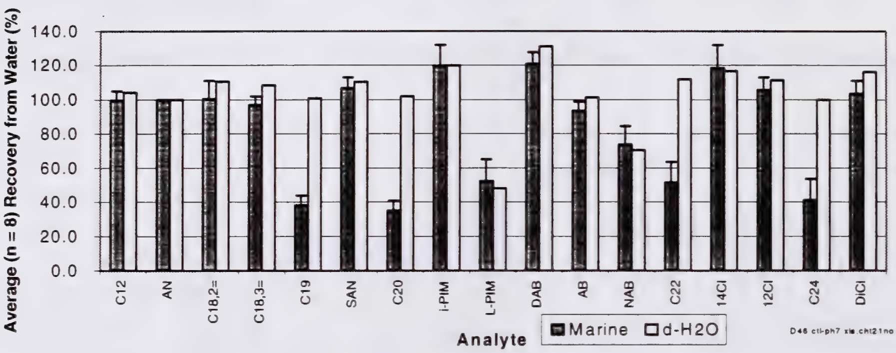

Figure 5. Recoveries of spiked RFAs from marine water at pH 7 using dichloromethane. Results for distilled water (Figure 3) replotted.

TRACE DETERMINATIONS OF ORGANOCHLORINES USING EMPORE DISC EXTRACTIONS AND GCMS

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INTRODUCTION AND SUMMARY OF METHOD

The City of Edmonton is required to monitor for trace organochlorines and other pesticides in drinking water . A method for determining trace quantities of organochlorines in drinking water using the Empore disc extraction and capillary gas chromatograph/mass spectrometric analysis was developed. It is patterned after EPA method 525.2.

This method involve adjusting the sample to pH less than 2 using 6N HCl. Surrogates and internal standard are added to the sample for monitoring losses and to minimize the possibility of false negative results. The sample is extracted using C_{18} Empore disks and eluted with methylene chloride. The methylene chloride extract is then dried, concentrated, and an internal standard added prior to extraction. This extract is injected into a capillary GCMS instrument in scan mode. A qualitative screening is performed using the relative retention time and relative abundance of two or more characteristic ions. Quantitative analyses of target compounds are performed using an internal standard technique in which the extracted areas of characteristic ions are utilized in the calculation.

EMPORE DISK EXTRACTION PROCEDURE

1. Set up extraction flask with C18 EMPORE extraction filter as shown in Figure 1

2. Transfer 1 L of sample into a 1L volumetric flask.

3. Check pH

4. If pH>2, add 1mL of 6N HCl, recheck pH to ensure that it is less than 2.

5. Transfer sample into a 1.2L bottle with Teflon lined cap.

6. Add 5 ml of pesticide grade methanol, 100 µL of internal standard-surrogate mixture to sample in bottle, screw on cap and shake to thoroughly mix contents.

7. Rinse extraction apparatus with EMPORE filter with 5 mL of methylene chloride. Allow methylene chloride to slowly drip through the filter for about 2-3 mins. Apply vacuum to draw methylene chloride completely through the disk for about 1 min.

8. Add about 2 mL of pesticide grade methanol to rinse methylene chloride out.

9. Add 8 mL of methanol onto the filter, apply vacuum until about 4 mL have passed through, then pour sample on the top and allow bottle to sit on top of the filter as shown in Figure 1

10. When all the sample has passed through, allow vacuum to continue drawing air through filter for 5-10 mins. This allows filter and apparatus to dry.

11. Empty the flask containing the extracted sample.

12. Attach the collection tube into the bottom of the filter base and sitting in the collection flask as shown in Figure 2.

13. Carefully transfer 10 mL methylene chloride by rinsing the inside edge of the filter funnel with a disposable pipette. Let the solvent collect and soak the filter for about 2-3 mins. Draw solvent through the filter slowly by applying half the full vacuum. When all the solvent has passed through the filter, continue applying the vacuum for another 1-2 mins.

14. Transfer the extract to a 25 mL erlenmeyer flask containing 3 gm of sodium sulfate. Sodium sulfate is used as a drying agent for the extract. Swirl the flask for 1-2 mins to facilitate the drying process. Transfer the dried extract through a 5 inch disposable pipette stuffed with 2 -3 cm of glass wool into a 50 mL pear shaped flask. The glass wool filters out any sodium sulfate that was transferred from the extract.

15. Repeat the extraction procedures described in 13 twice with 5 mL of methylene chloride and transfer the extract into the same collection tube used in 12 and repeat the procedures used in 14, collecting the two 5 mL aliquots into the same 50 mL pear shaped flasks used in 14.

Connect the pear shaped flask to the rotovac and reduce the solvent to about 0.1 to 0.4 mL. Add 0.2 to 0.5 mL of methanol to this solution and then transfer it to a 1mL volumetric flask. Rinse out pear shaped flask with two to three aliquots of 0.2 mL until the solution measures exactly 1 mL. Transfer this solution into an automatic sample vial and cover with cap.

FIGURE 1: EMPORE DISK EXTRACTION SET-UP

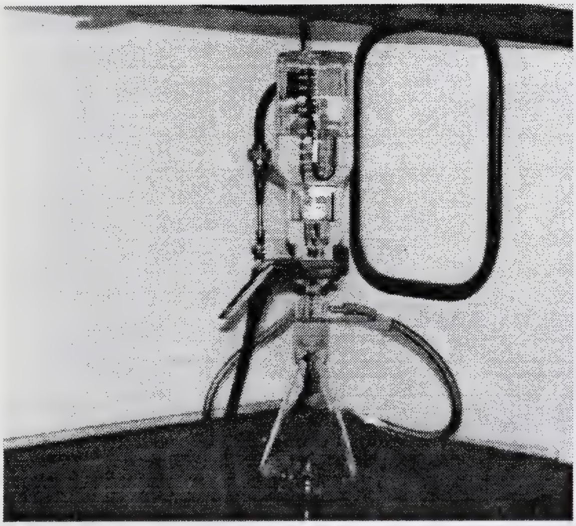
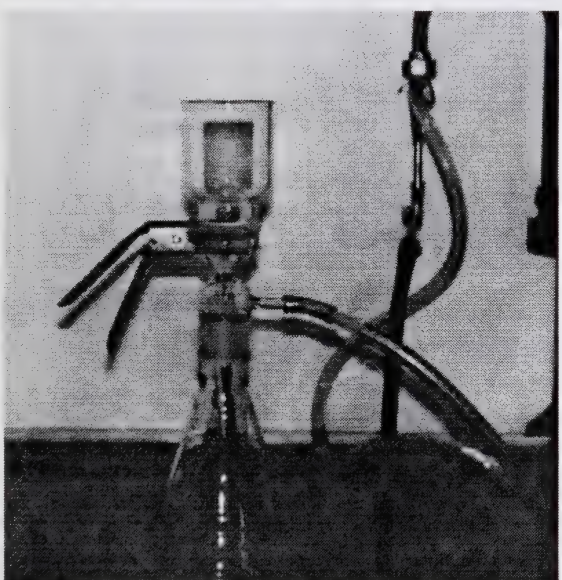


FIGURE 2: SET-UP AT SAMPLE COLLECTION STAGE



TUNING OF GCMS

Analysis with GCMS is carried out as per EPA protocol. A successful DFTPP (decafluorotriphenyl phosphine) tune must be obtained before analysis can be performed. A successful DFTPP tune is obtained when a solution of 10 μg/mL (50 ηg injected) DFTPP solution in methanol satisfies all the criteria seen in Table 1.

TABLE 1: CRITERIA FOR SUCCESSFUL GCMS OPERATION

Mass	Ion Abundance Criteria
51	30 to 60% of mass 198
68	less than 2% of mass 69
70	less than 2% of mass 69
127	40 to 60% of mass 198
197	less than 1 % of mass 198
198	base peak ,100% relative abundance
199	5 to 9% of mass 198
275	10 to 30% of mass 198
365	greater than 1% of base peak
441	present but less than mass 443
442	base peak or greater than 40% 198
443	17-23% of mass 442

Conditions of the GCMS were as shown in below:

INJECTOR INFORMATION

Inlet: GC	
Injector source	Auto
Injector location	Front
Sample Washes	3
Sample Pumps	4
Sample Volume	5 stop(s)
Viscosity delay	0
Solvent A washes	3
Solvent B washes	3
On column	No
Purge A	off at 0 time, on at 0.75 mins
On column	No

MASS SPECTROMETRIC ANALYTICAL PARAMETERS

Solvent delay	3.00 min
EM absolute	False
Tune File	DFTPP.U
Acquisition mode	Scan
Low Mass	41
High Mass	450
Sampling	3
A/D Samples	8
Threshold	500
Time Window	10 min
Total Ion Max	2,000,000

GC INFORMATION

Det A(not installed)	off
Det B	280°C
Inj A	250°C
Inj B(on-column)	250°C
Oven Equib Time.	0.5min
Oven Max	325°C
Cryo	off
Oven	on
Column Used	DB-5, 30m*0.25mm, film=0.25μm

GC TEMPERATURE PROGRAM

Initial Temp		70°C	
Initial Time		2 min	
Total run time		54.17 mins	
Level	Rate(°C/ min)	Final (°C)	Final Time (min)
1	12	180	5
2	12	215	3
3	12	240	30

TABLE OF RETENTION TIME, QUANTITATION IONS AND QUALIFIERS USED

COMPOUNDS	RT (mins)	QUANTITATION ION	QUALIFIER 1	QUALIFIER 2	QUALIFIER 3
Alachlor	19.85	160	45	188	
Aldrin	21.17	66	263	91	
Atrazine	16.95	200	215	58	
α-Chlordane	23.68	375	237	272	
γ-Chlordane	24.21	373	237		
Chlorpyrifos	21.36	197	314	97	
4,4'-DDE	24.86	246	248	176	
4,4'-DDD	26.39	235	237	165	
4,4'-DDT	28.03	235	237	165	
Dieldrin	24.95	79	263	108	
Heptachlor	19.89	100	272	274	
Heptachlor Epoxide	22.96	81	183	353	
Lindane	17.36	181	183		
Methoxychlor	31.19	227			
Simazine	16.76	201	186	173	96
trans-Nonachlor	24.38	409	407	411	
Acenapthene-d10	12.18	164			
Triallate	18.48	86	268	128	
Trifluralin	15.23	306	264		
Chrysene-d12	30.61	240			
Perylene-d12	47.70	264			

Method Validation

Method validation was performed by spiking certified standards containing analytes in concentrations between 1.6 μg/L and 10 μg/L into reagent water. Surrogates (acenapthene-d10, chrysene-d12 and perylene-d12) and internal standard (phenanthrene-d10) were added to the spiked samples and extracted and analysed as described.

RESULTS

TABLE 1: PRECISION AND ACCURACY DATA FROM 4 ANALYSES OF SPIKED 1 L OF REAGENT WATER

Compounds	SPIKED VALUE µg/L	AVERAGE µg/L	AVE RECOVERY (%)	STD DEV µg/L	MDL µg/L	RSD %
Acenaphthene-d10	5.00	4.10	82	0.75	4.4	18
Simazine	1.61	1.43	89	0.24	1.4	17
Atrazine	1.61	1.73	107	0.05	0.3	3
Phenanthrene-d10	5.00	5.00	100	0.00	0.0	0
Heptachlor	1.61	1.73	107	0.05	0.3	3
Alachlor	1.61	1.60	99	0.14	0.8	9
Aldrin	1.61	1.58	98	0.21	1.2	13
Heptachlor Epoxide	1.61	1.88	116	0.15	0.9	8
a-Chlordane	1.61	1.50	93	0.00	0.0	0
g-Chlordane	1.61	1.60	99	0.00	0.0	0
Trans-Nonachlor	1.61	1.90	118	0.00	0.0	0
Chrysene-d12	5.00	4.25	85	0.13	0.8	3
Methoxychlor	1.61	1.45	90	0.24	1.4	16
Perylene-d12	5.00	3.98	80	0.21	1.2	5

All MDLs calculated using EPA protocols :

MDL = S t (n-1, 1-α=0.99)

Where:

t(n-1, 1-α=0.99) =Student's t value for the 99% confidence level with n-1 degrees of freedom

n= number of replicates
S= the std deviation of replicate ANALYSES

TABLE 2: PRECISION AND ACCURACY DATA FROM ANALYSES OF SPIKED 1 L OF REAGENT WATER

COMPOUND	SPIKED VALUE µg/L	AVERAGE RECOVERY %	STD DEV µg/L	RSD %	MDL µg/L	NO. OF ANALYSES
Acenaphthene-d10	5.01	82	0.173	4	0.6	7
Trifluralin	2.48	87	0.243	11	1.0	6
Lindane	2.50	83	0.273	13	1.0	7
Trialliate	2.45	89	0.107	5	0.4	7
Heptachlor	**2.45***	**81**	**0.091***	**5**	**0.3***	**11**
Aldrin	**2.47**	**79**	**0.178***	**9**	**0.6***	**10**
Heptachlor epoxide	**2.47**	**95**	**0.066***	**3**	**0.2***	**11**
Chrysene-d12	5.01	104	0.564	11	2.1	7
Methoxychlor	**9.86**	**88**	**0.870***	**10**	**2.8***	**11**
Perylene-d12	5.01	100	0.970	19	3.6	7
4,4' DDE	**2.47**	**93**	**0.148***	**6**	**0.5***	**11**
4,4' DDD	**2.46**	**96**	**0.087***	**4**	**0.3***	**11**
4,4' DDT	**2.47**	**107**	**0.165***	**6**	**0.5***	**11**
Dieldrin	**2.46**	**91**	**0.096***	**4**	**0.3***	**11**

* Calculated from pooled std deviation

TABLE 3: PRECISION AND ACCURACY DATA FROM ANALYSES OF SPIKED 1 L OF REAGENT WATER

COMPOUND	SPIKED VALUE µg/L	AVERAGE RECOVERY µg/L	AVE % RECOVERY	STD DEV. µg/L	MDL µg/L	RSD %	NO.OF ANALYSES
Acenaphthene-d10(Surrogates)	5.00	5.04	101	0.322	1.3	6	6
Trifluralin	**5.01**	**5.42**	**108**	**0.363***	**1.1***	**6**	**12**
Simazine	5.00	4.30	86	0.683	3.1	16	5
Atrazine	5.00	5.47	109	0.803	3.7	15	6
Triallate	**5.00**	**5.43**	**109**	**0.171***	**0.5***	**3**	**12**
Alachlor	5.02	5.76	115	0.176	0.8	3	6
Heptachlor	5.00	5.20	97	0.180	0.7	3	6
Aldrin	5.00	4.22	84	0.613	2.5	15	6
Heptachlor epoxide	5.00	4.89	98	0.172	0.7	4	6
g-Chlordane	5.00	5.18	103	0.166	0.7	3	6
a-Chlordane	5.00	5.28		0.235	0.9	4	6
Trans-Nonachlor	5.00	5.78	116	0.240	1.0	4	6
Chrysene-d12(Surrogate)	5.00	4.90	98	0.214	0.9	4	6
Methoxychlor	5.00	6.18	123	0.170	0.7	3	6
Chlorpyrifos	5.00	4.93	99	0.234	0.9	5	6
Perylene-d12 (Surrogate)	5.00	5.00	100	0.239	1.0	5	6

* Calculated from pooled std deviation

TABLE 4 AVERAGE PERCENT RECOVERY OF SPIKES OF 5μg/L INTO 1L TAP WATER

COMPOUNDS	AVE % RECOVERY	STD DEV OF % RECOVERY	RSD %	NO. OF ANALYSES
Acenapthene d10	70	19	27	9
Alachlor	112	17	15	6
Aldrin	79	14	17	9
Atrazine	120	9	7	5
Chlordane (alpha)	104	11	10	6
Chlordane (gamma)	103	11	11	5
Chrysene-d12	110	11	10	8
DDD	104	5	4	4
DDE	93	15	16	4
DDT	114	7	6	4
Dieldrin	104	4	4	4
heptachlor	108	12	11	9
Heptachlor Epoxide	106	12	12	9
Lindane	135	34	25	8
Methoxychlor	129	26	20	7
Perylene- d12	102	12	12	9
Simazine	103	12	12	5
trans-Nonachlor	112	8	7	5
Triallate	90	20	23	5
Trifluralin	98	15	15	4

CONCLUSION

The average percent recoveries from method validation using this procedure were good ranging from 79% to 123%. The majority of analytes(96%) have recoveries between 80% to 120%. The MDL from the majority of the compounds were reasonably good with less than 1 μg/L. There were a few exceptions with high MDL from simazine (1.4 to 3.1 μg/L), atrazine (0.3 to 3.7 μg/L), aldrin (0.6 to 2.5 μg/L) and methoxychlor (0.7 to 2.8 μg/L). These high values were obtain with spikes at 5 and 10 μg/L. The MDL obtained for these compounds at low level spikes were good. The recoveries and relative standard deviation for all the compounds tested fall within US EPA acceptable criteria of recoveries of 70-130% and RSD < 30%. These results shows that this method can be use for analysing many organochlorines, atrazine, simazine, chlorpyrifos, trifluralin and triallate.

Spikes of 5 μg/L in tap water matrix showed a bias towards high recoveries and standard deviations for recoveries of lindane (R=135%, SD= 34%) and methoxychlor (R=129%, SD= 26%). Spikes of lindane in reagent water does not show this bias. For methoxychlor, only the 5μg/L spike in reagent water showed a similar bias to high recoveries while spikes at 1.6 and 10 μg/L did not show this trend.

SAMPLING FOR ENVIRONMENTAL COMPLIANCE VERIFICATION

Tim Lambert

Regulatory Services & Coordination, Environmental Protection, Environment Canada
Edmonton, AB T6B 2X3

The collection of environmental samples is a critical step in compliance verification. There exists the potential for any collected sample to be an exhibit in a court prosecution for violation of environmental regulations. Thus it is critical that samples are collected properly following established protocols. Environment Canada has established protocols for the collection of samples based on current practices for the collection of samples, along with guidelines for submitting the samples to the laboratory. With the growing number of environmental regulations controlling toxic substances, both enforcement staff and analysts are increasingly faced with the challenge of safely sampling or analyzing an unknown substance or waste.

SAMPLE PREPARATION USING ACCELERATED SOLVENT EXTRACTION

(U.S. EPA METHOD 3545, PROPOSED)

BY

KHAM LIN

DIONEX CANADA LTD.

ASE
Accelerated Solvent Extraction

ASE 200

DIONEX

Sample Extraction Techniques

- Soxhlet
- Automated Soxhlet
- Sonication
- Microwave
- Supercritical fluid
- Solid phase
- Liquid-solid
- Liquid-liquid
- Purge and trap

What if We Used Hot Liquid Solvents?

- Favorable physical properties
  - Viscosities and diffusivities
  - Solvent strengths
  - Desorption and solubilization kinetics
  - No phase change for trapping
- Easier to overcome matrix effects
- Easier to overcome intermolecular forces
- Higher solubility
  - Anthracene: 15 fold increase, 50-150 °C

11012

Introducing a New Sample Preparation Technique: Accelerated Solvent Extraction (ASE™)

Uses elevated temperatures and pressures with organic solvents to achieve fast and efficient sample extractions.

11010

Effect of ASE™ Operational Parameters

- Temperature
  - Major impact on recoveries
    Diffusion rates
    Viscosities
    Energy of desorption
    Mass transfer
    Increased solubility
  - Typical values: 100-150 °C (200 °C max)

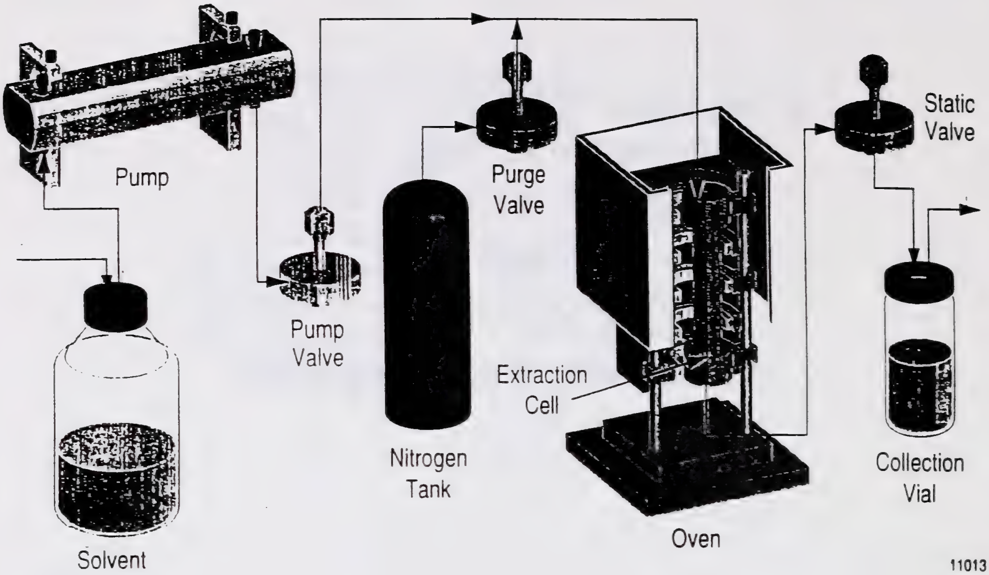
11018

Effect of ASE™ Operational Parameters

- Pressure
  - Seems to have limited effect on recovery with samples studied
  - Pressure needed to keep solvents liquid at high temperatures
  - Cells fill faster with high pressures
  - Typical values: 1200-2500 psi (3000 psi max)

11017

ASE™ Schematic I



Typical Times and Volumes Used in ASE™

- Volume of solvent used
  - 10 mL cell: 12-15 mL
  - 20 mL cell: 25-30 mL
  - 30 mL cell: 35-45 mL
- Time required (excluding loading)
  - Filling cell with solvent: 20-60 sec
  - Thermal equilibration: 5 min
  - Static period: 5 min
  - Solvent flush: 20-60 sec
  - Gas purge: 30-90 sec
- Total time: 12-14 min

11016

Comparison of Liquid Extraction Techniques

Technique	Sample Size (g)	Solvent Vol. (mL)	Sample/Solvent (x10)
Soxhlet	10 - 30	300 - 500	0.33 - 0.60
Sonication	30	300 - 400	0.75 - 1.00
Microwave	5	30	1.67
Shake	50	300	1.67
Automated Soxhlet	10	50	2.00
ASE™	10 - 30	15 - 45	6.67

11019

How Does the Performance of ASE™ Compare to Standard EPA Extraction Procedures?

- Soxhlet: EPA Method 3540
- Automated Soxhlet: EPA Method 3541
- Shake Extraction: EPA Method 8150A

11029

ASE™ Comparison Study Experimental Design

- Organochlorine pesticides (OCP): 20 compounds, 3 levels
- Semivolatiles (BNA): 56 compounds, 3 levels
- Organophosphorus pesticides (OPP): 24 compounds, 2 levels
- Herbicides: 8 compounds, 2 levels
- Three matrices: clay, loam, sand
- Standard EPA extraction procedures done in parallel

11030

Extraction of BNA Conditions Used

	ASE™	Automated Soxhlet
Sample size	10 or 14 g	10 or 14 g
Solvent	Methylene chloride/acetone (1:1), 14 mL	Methylene chloride/acetone (1:1) 50 mL
Temperature	100 °C	140 °C on heater
Pressure	2000 psi	Atmospheric
Time	5 min equilibration, 5 min static	60 min boil, 60 min rinse
Analytical	GC-MS	GC-MS

11075

ASE™ Comparison Study Results Summary for BNA

- 99.2% average relative recovery of all compounds at all concentrations and from all matrices
- 12.8% RSD for ASE™, 13.9% for Automated Soxhlet
- ASE vs. Automated Soxhlet linearity (504 data points)
  - Slope: 0.966
  - Intercept: 51.5
  - r^2: 0.977

11077

Extraction of OCP Conditions Used

	ASE™	Automated Soxhlet
Sample size	10 g	10 g
Solvent	Hexane/acetone (1:1), 14 mL	Hexane/acetone, 50 mL
Temperature	100 °C	140 °C on heater
Pressure	2000 psi	Atmospheric
Time	5 min equilibration, 5 min static	60 min boil, 60 min rinse
Analytical	GC-ECD	GC-ECD

11033

ASE™ Comparison Study
Results Summary for OCP

- 97.3% average relative recovery of all compounds at all concentrations and from all matrices
- 8.3% RSD for ASE, 8.7% for Automated Soxhlet
- ASE vs. Automated Soxhlet linearity (180 data points)
  – Slope: 0.959
  – Intercept: -0.889
  – r^2: 0.971

11035

ASE™ Comparison Study
Results Summary for OPP

- 98.6% average relative recovery of all compounds at all concentrations and from all matrices
- 9.3% RSD for ASE, 8.4% for Soxhlet
- ASE vs. Soxhlet linearity (144 data points)
  – Slope: 0.961
  – Intercept: 12.8
  – r^2: 0.993

11038

*A*SE EPA Acceptance

"…. an automated microextraction technique developed by Dionex as Method 3545 - Accelerated Solvent Extraction (*A*SE). This method is applicable to virtually all of the extractable organics on the RCRA target analyte list."

Barry Lesnik - National Organic Program Mgr.
RCRA Program U.S. E. P. A. Office of Solid Waste
Environmental Lab Dec/Jan 94/95 pg 27

Extraction of PAH by ASE™ Conditions Used

- 0.5 - 20 g Sample
- Methylene chloride, toluene/methanol or methylene chloride/acetone
- 100 °C
- 2000 psi
- 5 min equilibration, 5 min static
- 5 - 30 mL solvent HPLC and GC-MS analysis

Extraction of PAH by ASE™
Recovery from Contaminated Soil*

Compound	Avg. (%), n=8	RSD (%)
Fluorene	83.4	1.6
Phenanthrene	119.2	1.9
Anthracene	88.0	6.6
Fluoranthene	101.2	14
Pyrene	104.8	18
Benz[a]anthracene	93.6	10
Chrysene	121.8	15
Benzo[b+k]fluoranthene	142.3	8.1
Benzo[a]pyrene	100.3	15

* Fisher SRS 103-100 (20-1400 mg/kg)

11065

Extraction of PCB by ASE™
Conditions Used

- 0.5 - 5 g sample
- Iso-octane or hexane/acetone
- 100 °C
- 2000 psi
- 5 min equilibration, 5 min static
- 6 -15 mL solvent, GC analysis

11020

Extraction of PCB by ASE™ Recovery* from Sewage Sludge**

PCB Congener	Avg. (%), n=6	RSD (%)
PCB 28	118.1	2.5
PCB 52	114.0	4.7
PCB 101	142.9	7.4
PCB 153	109.5	5.8
PCB 138	109.6	3.9
PCB 180	160.4	7.5

* As compared to Soxhlet

** Sample from Germany (160-200 μg/kg)

11021

ASE™ Comparison Study Conclusions

- ASE is equivalent to EPA extraction techniques for these compound classes from solid and semi-solid matrices
  - Organochlorine pesticides (OCP)
  - Organophosphorus pesticides (OPP)
  - Herbicides
  - Semivolatiles (BNA)
  - Polychlorinated biphenyls (PCB)
  - Polycyclic aromatic hydrocarbons (PAH)

11042

ASE™ Comparison Study Conclusions (continued)

- Based on results of study, ASE has been accepted and proposed as EPA Method 3545 by the SW-846 Committee for the following compound classes:
  - OCP, OPP, Herbicides, BNA, PCB and PAH
- Proposed Method 3545 scheduled to be included in update III of US EPA Solid Waste Methods in 40CFR

11043

Conclusions

- ASE™ is faster than conventional liquid extraction procedures
  - <15 min vs. 2-24 h
- ASE uses less solvent than conventional liquid extraction procedures
  - <15 mL for 10 g sample vs. 50-500
- ASE does not have matrix dependence as SFE
- ASE uses same solvents as used in conventional procedures
  - Method development is greatly simplified

11044

ANALYSIS OF CLOFENTEZINE IN APPLES AND PEARS

BY HPLC/UV AND GC/MS

BY

RALPH HINDLE

HEALTH & WELFARE CANADA

Analysis of Clofentezine by HPLC With UV Detection

1. Scope

This method has been applied to apples and pears, with a limit of detection of less than 0.05 parts per million (ppm) for a 30 g sample. The Maximum Residue Limit (MRL) at the time of this writing is 0.5 ppm for both apples and pears. The method can be used for rapid screening of samples for the parent compound, clofentezine. In order to establish compliance with the Regulations of the Food and Drug Act, which requires determination of the parent and all metabolites containing the 2-chlorobenzoyl moiety, it is necessary to use a method which hydrolyzes all compounds to 2-chlorobenzoic acid. Hydrolysis methods are not included in this procedure.

2. References

1. J.D. Manley and P.J. Snowdon; Analytical Method for Residues of Clofentezine in Miscellaneous Fruit Crops. *FBC Limited Method RESID/86/48*, **1986**. Provides general analytical approach and UV wavelength.

2. Pree, D. J.; Marshall, D.B.; McGarvey, B.D.; Residual Toxicity of Dicofol, Formetanate HCl, Propargite, Hexythiazox, and Clofentezine to European Red Mite on Peach. *The Canadian Entomologist*, **1992**, 59-67. Provides background on pest control, HPLC conditions, and persistence data.

3. Fillion, J.; Hindle, R.; Lacroix, M.; Selwyn, J.; Multiresidue Method for the Determination of Pesticides in Fruit and Vegetables by GC/MS and HPLC/Fluorescence. *J. AOAC Int.* in publication (accepted March 1, 1995). Provides extraction procedure.

4. Bicchi, C. and D'Amato, A.; Simultaneous Determination of Clofentezine, Fenoxycarb and Hexythiazox by HPLC on Apples, Pears and Their Pulps. *Pestic. Sci.*, **1990**, 13-19. Provides Sep-Pak cleanup and HPLC conditions.

3. Principle

Clofentezine is extracted into acetonitrile, separated from the aqueous phase by addition of sodium chloride, concentrated, filtered, and analyzed by reverse-phase HPLC with UV detection. An optional Sep-Pak cleanup step can be performed on the concentrated extract prior to HPLC analysis.

4. Reagents, Solutions, and Standards

Analytical standard: clofentezine, Food Research Division, Ottawa. Prepare a stock solution by dissolving 2.5 mg in acetone, and make to volume in a 25-mL volumetric flask. A 1-μg/mL clofentezine solution is prepared by diluting 1 mL of the stock to 100 mL with acetone. This solution is used to spike the fortified samples.

Acetone and acetonitrile (CH_3CN): distilled in glass.

Extraction Solvent: 100 mL CH_3CN.

5. Equipment and Materials

Robot Coupe R6N

Polytron: Kinematica GmbH.

Centrifuge: International Centrifuge Model EXD.

Cleanup columns: Waters C18 Sep-Pak 12cc cartridges (2g); Part No. 36915.

Sample filters: Gelman Acrodisc 13, PTFE 0.45μ

HPLC:	Hewlett-Packard 1090M Series II with autosampler, 100μL injection loop, Pascal data system.
Detector:	Hewlett-Packard Model 1046A, λ = 270 nm.
Column:	Supelco 25 cm x 4.6 mm Supelcosil LC-8, 5 μm.
Solvents:	A = CH_3CN B = H_2O
Flow:	1.0 mL/min., 70% A, 30% B.

6. Sample Preparation

Quarter and dice the apples, then homogenize using the Robot-Coupe. Transfer unused portion to a mason jar for freezing and storage.

7. Procedure

7.1 Extraction

Weigh 30 g of the homogenate into a glass centrifuge bottle.

Add 100 mL of CH_3CN to the centrifuge bottle.

Polytron the sample for 1 minute.

Add 1 tsp. NaCl.

Polytron for 1 minute.

Transfer 13 mL to a clean 15-mL graduated centrifuge tube.

Add Na_2SO_4 until volume reads 15 mL. Shake tube vigorously for 10 seconds.

Centrifuge for 1 minute.

Using a pasteur pipette, transfer 5 mL to a clean 15-mL centrifuge tube.

7.1.1 Cleanup

Note: *Pears do not show an interference with clofentezine, and may not require this cleanup. Apples, on the other hand, will benefit from cleanup.*

Reduce the volume to 2 mL under nitrogen.

Pre-wash Sep-Pak cartridge with 5 mL CH_3CN.

Transfer sample to Sep-Pak, and allow sample to elute on to column. Collect eluate in a 15-mL centrifuge tube.

Elute column with 10 mL CH_3CN and collect in the same 15-mL centrifuge tube.

7.1.2 Preparation of Final Extract

Reduce the volume to 0.5 mL under nitrogen.

Adjust sample volume to 0.7 mL with CH_3CN and vortex. Adjust volume to 1.0 mL with H_2O and vortex.

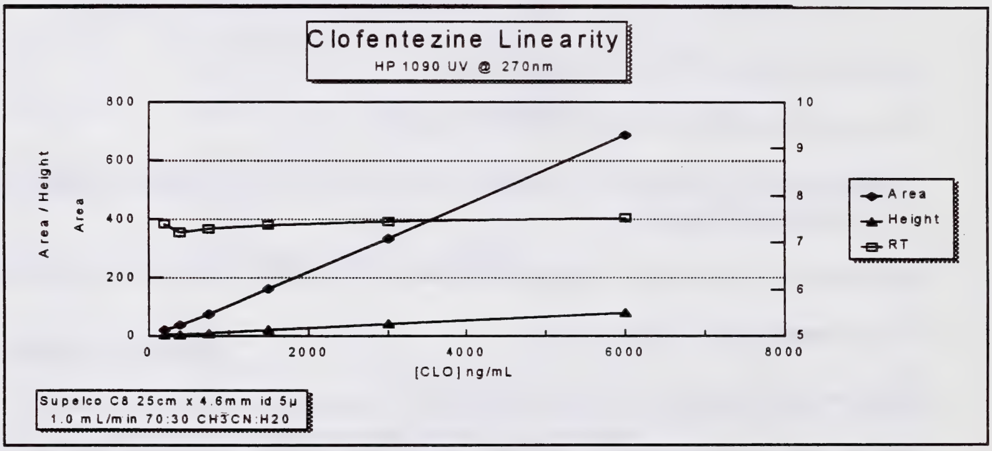
Filter sample through Gelman Acrodisc 13, PTFE 0.45μm, and collect eluate directly into HP autosampler vial for HPLC analysis.

8. Calibration and Calculations

Prepare standards to check linearity by pipetting 8.0 mL of a 3.0-μg/mL solution in acetone into a 15-mL centrifuge tube. Evaporate to dryness under nitrogen, and reconstitute with 4.0 mL HPLC mobile phase. Prepare serial dilutions by diluting 2 mL of this standard with 2 mL mobile phase and mixing. Repeat the dilutions with each subsequent solution as often as required to cover the desired range.

Clofentezine has been found to be linear with a near-zero-intercept in the concentration range of 190 to 6,000 ng/mL (30 μL injections):

File	[CLOF] (ng/mL)	Area	Height	RT
CLOFA63A	190	19.0	2.5	7.39
CLOFA64A	375	36.6	5	7.21
CLOFA65A	750	77.1	10	7.28
CLOFA66A	1500	161	22	7.37
CLOFA67A	3000	335	41	7.46
CLOFA68A	6000	686	80	7.53



To calculate the level of clofentezine in samples, use the following equation:

$$[clof]_{ppm} = \frac{Area_{spl}}{Area_{std}} \times [clof]_{std} \times \frac{100mL}{Wgt_{spl}} \times \frac{1mL}{5mL}$$

9. Example Chromatograms

1: clofentezine standard in HPLC mobile phase; 0.15 μg/mL; RT = 6.745
2: pear spiked at 0.05 ppm clofentezine; 1.5 g/mL
3: pear blank; 1.5 g/mL

```
1: LC A 270,4   440,50      of CLO_N80A.D
2: LC A 270,4   440,50      of CLO_060A.D
3: LC A 270,4   440,50      of CLO_061A.D
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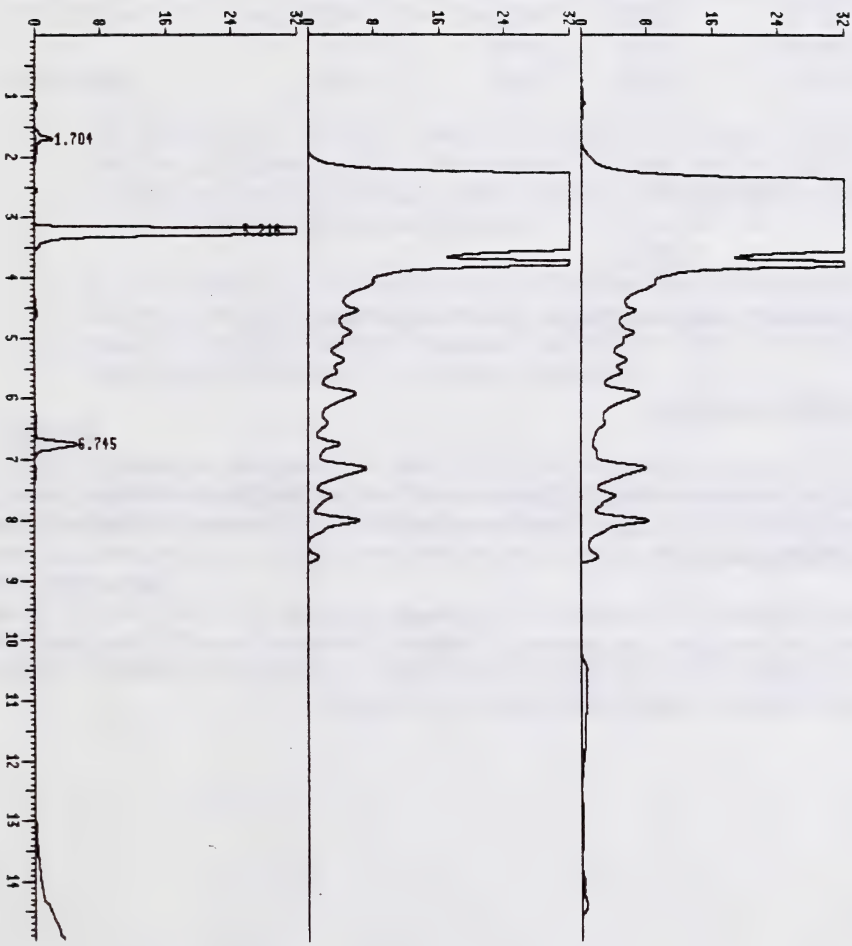


End of plot. Time = 0.01 to 14.97 minutes Chart speed = 1.29 cm/min

10. Recoveries

The following recoveries were obtained:

Date	Spike	Matrix	Recovery
21-Mar-95		solvent*	102.0%
21-Mar-95	0.50	pears	119.0%
23-Mar-95	0.50	pears	109.0%
23-Mar-95	0.10	pears	110.0%
23-Mar-95	0.10	pears	110.0%
28-Mar-95	0.05	pears	92.2%
28-Mar-95	0.05	pears	100.7%

Mean	106.8%
SD	8.41%
CV	7.87%

*Note: solvent recovery not included in calculations.

11. Critical Control Points

The initial extraction volume of CH_3CN must be measured accurately, since it separated from the water by salting out. All of the CH_3CN is separated, and therefore the final calculations depend upon the initial weight of sample in the extraction volume.

The final solvent composition of the sample must be 70:30 CH_3CN:H_2O, since that is how the standards are prepared. Different solvent ratios will affect peak shapes and possibly retention times. For this reason, it may be more reliable to perform the calculations based upon peak area and not height.

Analysis of Clofentezine with GC/MS by Hydrolysis to 2-Chlorobenzoic Acid

1. Scope

This method is applicable to the analysis of clofentezine and major metabolites in apples and pears, by hydrolysis of all components to a common moiety, namely 2-chlorobenzoic acid (2-CBA). Derivatization of 2-CBA to the methylated product allows analysis by GC/MS. References also indicate that the method is applicable to residues in bovine urine, tissues, and milk. This method allows enforcement of MRL's listed in the Regulations of the Food and Drug Act.

2. References

1. J.D. Manley, M.H. Peatman, and P.J. Snowdon; Analytical Method for Residues of Clofentezine and Metabolites in Animal Tissues and Milk. *FBC Limited Method RESID/85/32*, **1985**. Provides analytical approach to hydrolysis/extraction and derivatization.

2. P.J. Snowdon, R.J. Whiteoak, and J.D. Manley; The Hydrolysis of Clofentezine and Related Tetrazines as the Basis of Determination of Residues in Bovine Tissues. *Fresnius J Anal Chem*, **1991**, 444-447. Discusses mechanism of clofentezine hydrolysis.

3. Principle

Clofentezine and related tetrazine metabolites are liberated from conjugation and hydrolyzed to 2-chlorobenzoic acid by refluxing with concentrated HBr for 2 hours. Residues are partitioned into dichloromethane and methylated, with final analysis by GC/MS in scan mode.

4. Reagents, Solutions, and Standards

Analytical standards were supplied by Food Research Division, Ottawa.

Hydrobromic acid, 48%.

Hydrochloric acid

Sodium hydroxide

Ethyl acetate, distilled in glass.

Dichloromethane, distilled in glass.

Prepare a 100 μg/mL stock solution of 2-chlorobenzoic acid (2-CBA) by dissolving 5.0 mg of neat material in ethyl acetate, and diluting to 50.0 mL.

Prepare a 3 μg/mL spiking solution of 2-CBA by diluting the stock 3:100 in acetone.

5. Equipment and Materials

Robot Coupe R6N

Buchner funnels

Celite

Whatman No. 54 filter paper

6. Sample Preparation

Quarter and dice the apples, then homogenize using the Robot-Coupe. Transfer unused portion to a mason jar for freezing and storage.

7. Procedure

7.1 Extraction

Weigh 10 g of the homogenate into a 250-mL boiling flask.

For spiked samples, pipette 1.0 mL of the clofentezine spiking solution into the homogenate at this point.

Add 50 mL HBr to the flask, and a few boiling chips.

Reflux for 2 hours.

After the samples have cooled, filter under suction through Celite filtering aid and Whatman No. 54 filter paper.

Rinse the boiling flask 3 times with 25 mL distilled water, passing all eluates through the celite.

Transfer the filtrate into a 250-mL separatory funnel.

Re-rinse the boiling flask and celite with a total of 50 mL DCM, and transfer filtrate to the separatory funnel.

Partition and allow phases to separate. Collect DCM into a clean boiling flask.

Repeat partitioning with a further 50 mL DCM. Collect DCM in same flask as the first DCM fraction. Discard the aqueous phase.

Transfer DCM back into the separatory funnel. Rinse flask with 50 mL NaOH, and combine with DCM.

Partition and allow phases to separate. Discard lower DCM phase.

Acidify alkaline sample in separatory funnel with 25 mL 1N HCl.

Partition twice with 25 mL DCM, and discard aqueous layer. Pass each DCM phase through a pre-rinsed Na_2SO_4 column into a 250-mL boiling flask.

Reduce the volume to approximately 2 mL on a rotary evaporator.

Transfer the extract to a 15-mL centrifuge tube with DCM.

7.2 Derivatization

Reduce the DCM to just to dryness under a gentle stream of nitrogen at 40°C.

Add 2 mL ethereal diazomethane and allow reaction to proceed at room temperature for 30 minutes.

Evaporate just to dryness under a gentle stream of nitrogen at 40°C, and reconstitute in 2.0 mL ethyl acetate. The sample is now ready for GC analysis.

8. Calibration and Calculations

With each batch of samples, prepare a calibration standard by pipetting 1.0 mL of the spiking solution into a centrifuge tube and evaporate almost to dryness. Methylate the standard with 2 mL diazomethane for 30 minutes at room temperature, then evaporate the ether under nitrogen. Reconstitute in 2.0 mL ethyl acetate. The final concentration of the standard is 1.5 μg/mL.

Results are calculated by external standardization.

9. Example Chromatograms

to be added after GC runs made on Trio. All previous runs were on a Hewlett-Packard MSD.

10. Recoveries

Recovery of clofentezine spikes at 0.3 ppm was 59.3% ± 5.90% (CV = 9.95%, N = 4).

A single recovery of 2-CBA spiked at 0.3 ppm was 107%.

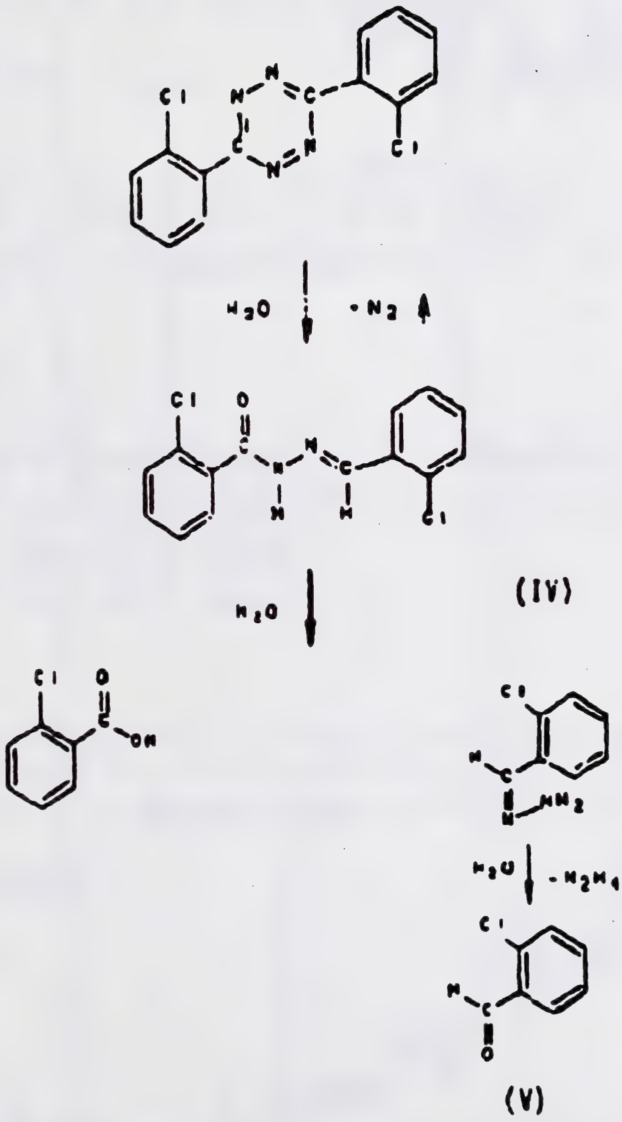
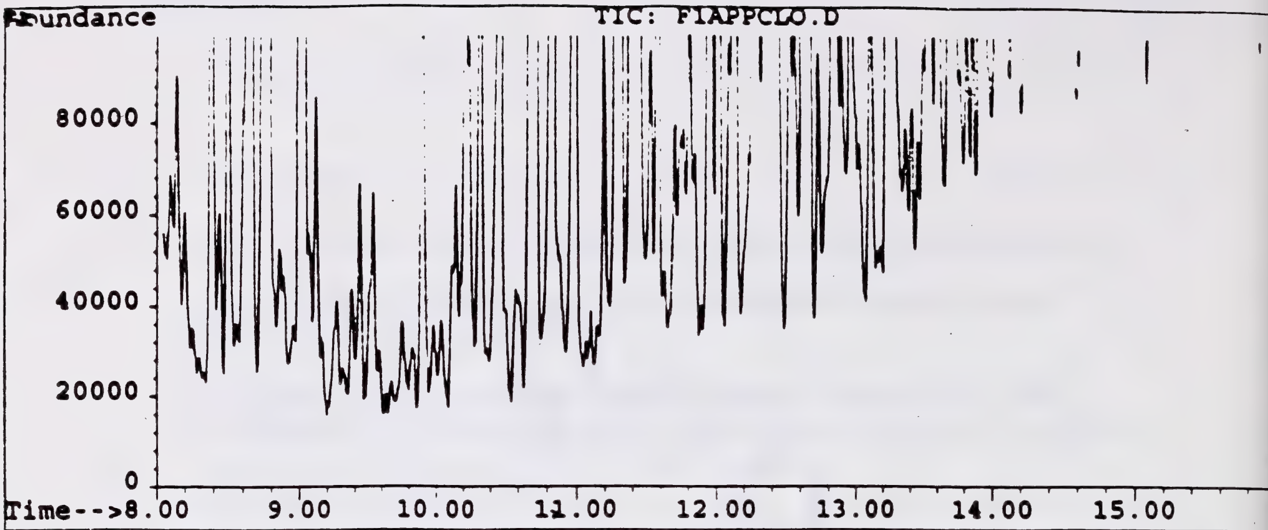


Fig. 2. Proposed mechanism for clofentezine hydrolysis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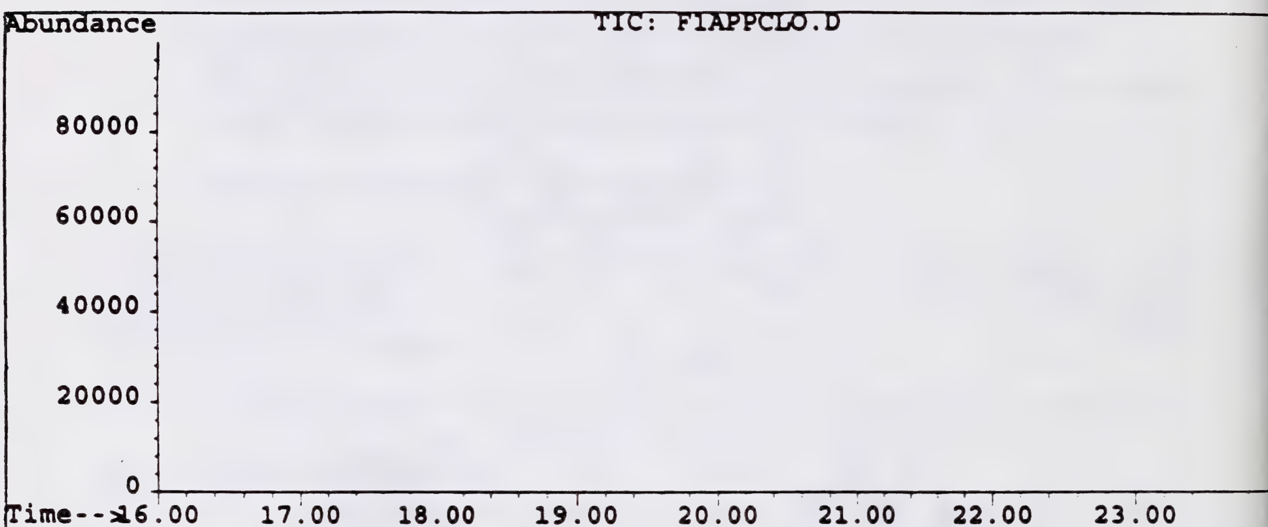
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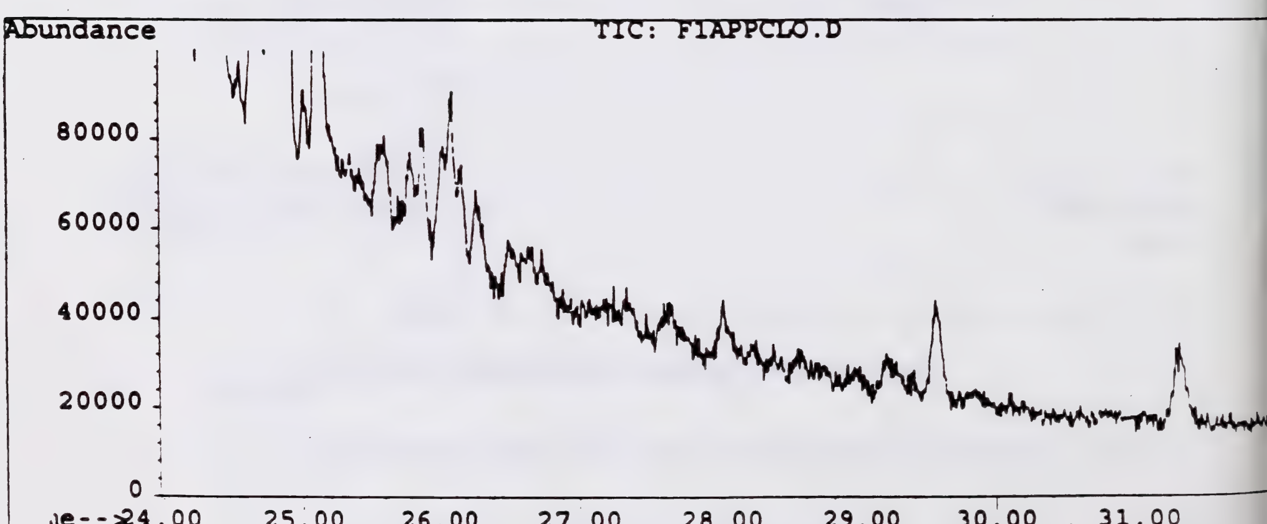
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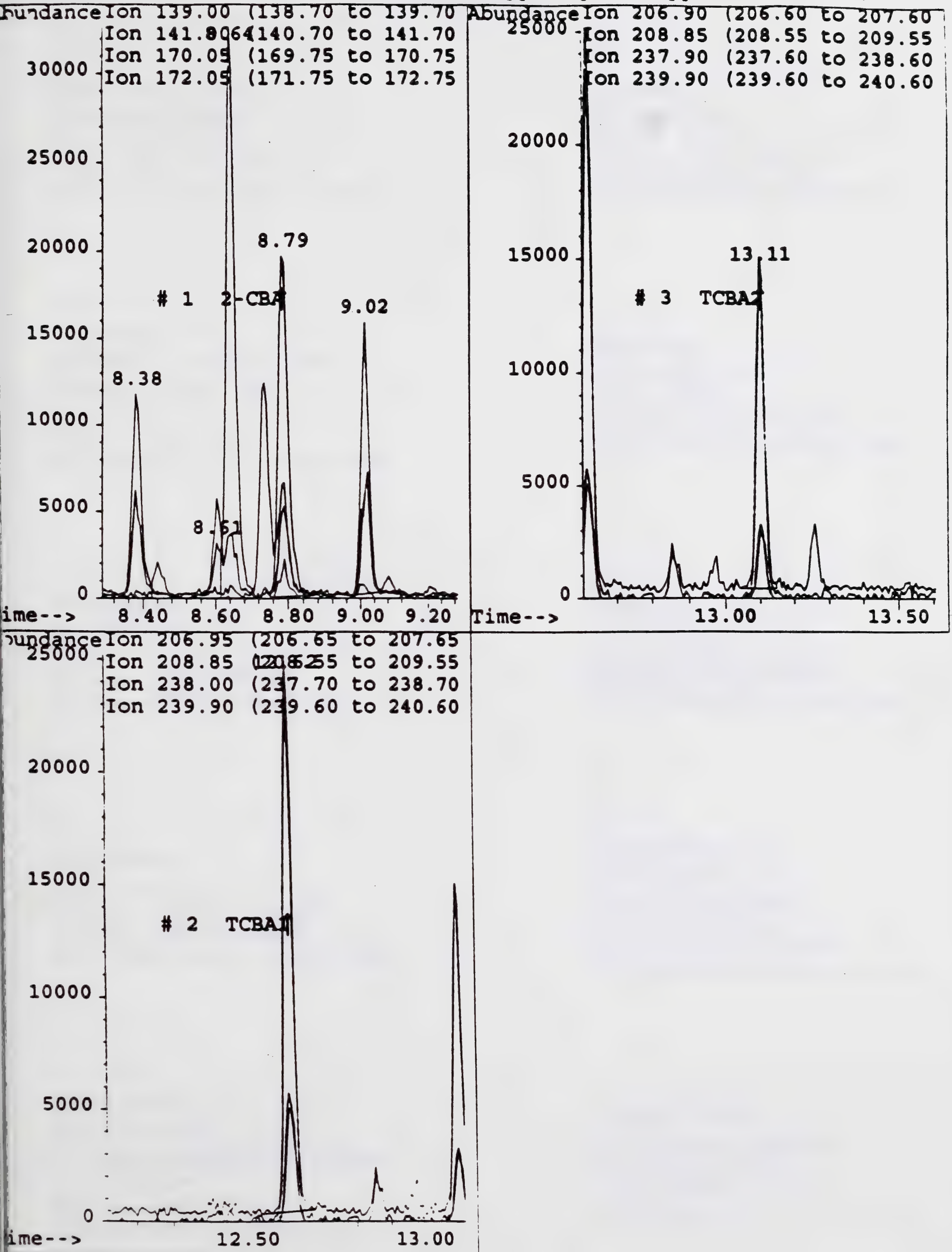
:\HPCHEM\1\DATA\OCT3VMIX\F1APPCLO.D Apple spike .3ppm clofentezine



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